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Method Development for Determination of Polychlorinated Hydrocarbons in Municipal Sludge

Southwest Research Inst.
San Antonio, TX

Prepared for

Municipal Environmental Research Lab.
Cincinnati, OH

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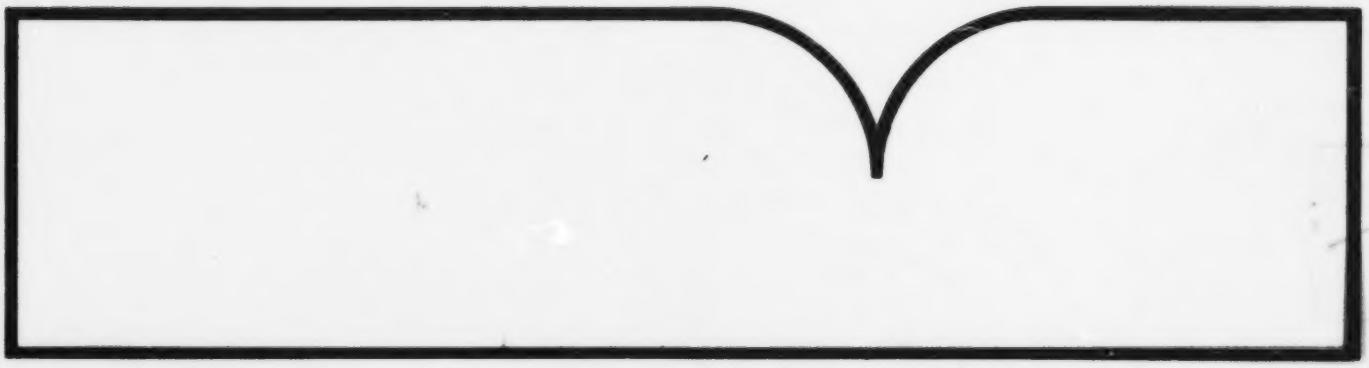

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Method Development for Determination of Polychlorinated Hydrocarbons in Municipal Sludge

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16. ABSTRACT

The method provides a procedure for analysis of pesticides and PCB's in municipal sludge. The method includes extraction by a centrifuge technique of the chlorinated compounds from the sludge matrix; clean-up of the extract to remove interferences by sulfur precipitation as mercury sulfide, and by gel permeation or florisil chromatography; quantitation of the chlorinated compounds by an electron capture detector with GC chromatography; and confirmation of the chlorinated compounds by GC/MS/computer. The method provides confirmation of single component pesticides at 0.3 mg of pesticide per Kg of sludges. The recommended extracting solvent is 15% methylene chloride, 2% acetone and 83% hexane.

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METHOD DEVELOPMENT FOR DETERMINATION OF POLYCHLORINATED HYDROCARBONS IN MUNICIPAL SLUDGE

by

Charles F. Rodriguez
William A. McMahon
Richard E. Thomas
Southwest Research Institute
San Antonio, Texas 78284

Contract No. 68-03-2606

Project Officers

James E. Longbottom
Physical & Chemical Methods Branch
Environmental Monitoring and Support Laboratory
Cincinnati, Ohio 45268
and
Dolloff F. Bishop
Wastewater Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

ENVIRONMENTAL MONITORING AND SUPPORT LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution and it involves defining the problem, measuring its impact, and searching for solutions. The Environmental Monitoring and Support Laboratory-Cincinnati conducts research to:

> Develop and evaluate techniques to measure the presence and concentration of physical, chemical, and radiological pollutants in water, wastewater, bottom sediments, and solid waste.
>
> Investigate methods for the concentration, recovery, and identification of viruses, bacteria, and other microbiological organisms in water and to determine the responses of aquatic organisms to water quality.
>
> Develop and operate an Agency-wide quality assurance program to assure standardization and quality control of systems for monitoring water and wastewater.

The Municipal Environmental Research Laboratory develops new and improved technology and systems for the prevention, treatment, and management of wastewater and solid and hazardous waste pollutant discharges from muncipal and community sources, for the preservation and treatment of public drinking water supplies, and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research; a most vital communications link between the researcher and the user community.

The report describes the development of a method for measuring the amounts of pesticides and PCB's in municipal sludge. The method uses GC quantitation with an electron capture detector and confirms the identity of the organics with GC/MS techniques.

Dwight G. Ballinger, Director
Environmental Monitoring & Support Laboratory

Francis T. Mayo, Director
Municipal Environmental Research Laboratory

ABSTRACT

The main objective of this program is development and evaluation of an analytical method for the determination of polychlorinated pesticides and biphenyls in the sludge from municipal sewage treatment plants.

The method provides a reliable means of:

1. separating the chlorinated compounds from the sample matrix
2. isolating the chlorinated compounds from interferences
3. concentrating the chlorinated compounds to within the detectability range of the determination method
4. separating and detecting the individual compounds, and
5. confirming the identity of the individual compounds.

Liquid-liquid partitioning followed by centrifugation to break the resultant emulsion is the method of separating the chlorinated compounds from the sludge. Elemental sulfur is removed by precipitation as mercury sulfide. Enough background interference is removed by either Florisil chromatography or steric exclusion chromatography to allow quantitative determinations by gas chromatography and electron capture detection. Confirmation of identity of the chlorinated compounds is made by gas chromatography/mass spectrometry. The minimum detectability level for the method is 0.3 μg of each single component pesticide per gram of dry sludge solids at a demonstrated level of accuracy and reliability.

The method was tested with 15 individual pesticides: aldrin, α-, β-, γ- and δ-BHC, DDD, DDE, DDT, dieldrin, endosulfan I and II, endrin, endrin aldehyde, heptachlor and heptachlor epoxide; two multicomponent pesticide formulations, chlordane and toxaphene; and one polychlorinated biphenyl, Aroclor 1260.

Initial methods development work was done by spiking sludge obtained from the San Antonio Rilling Road Sewage Treatment Plant with known quantities of the study compounds. After the method was established, it was evaluated with respect to applicability, accuracy and precision by determining known amounts of compounds spiked at two concentration levels in sludges from Dayton and Cincinnati, Ohio treatment plants.

This interim report was submitted in partial fulfillment of Contract No. 68-03-2606 by Southwest Research Institute under the sponsorship of the U. S. Environmental Protection Agency. This report covers the period 1 June 1978 to 31 December 1978.

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SECTION 1

INTRODUCTION

Since 1969 the number of pesticide formulations and their chemical constituents has continued to increase. The number has risen from 30,000 formulations of appxoximately 1000 substances to over 40,000 formulations of over 1800 substances listed by the Environmental Protection Agency (EPA). Because of the persistence of certain of the chlorinated pesticides and the indiscriminate toxicity displayed toward some nontarget species once they have been introduced into the environment, the use of these substances has been prohibited. The ban took effect in 1972 with DDT and has since then included compounds such as aldrin, dieldrin, chlordane, and heptachlor.

Because of the resistance to degradation that these chlorinated compounds display and the tremendous quantities that have been produced - about 1.6 billion pounds of pest control chemicals in 1975 in the U. S. alone - many of these compounds, introduced soon after World War II and banned in the period since 1972, may still be found in significant quantities in the environment. Notable among these compounds is DDT, probably the most widespread pesticide ever used. With the exception of 2,4,D, a herbicide, no other compound has approached the amounts of DDT used over the years. To aid the farmer to provide sustenance for the continually growing world population, it is necessary that pest control chemicals continue to be used, albiet in a correct and controlled manner.

As a part of its mission, the EPA has the tasks of monitoring the levels at which these various substances exist and enter the environment, controlling these levels to minimize toxic effects on nontarget species, and regulating the use of these substances so they may continue to be beneficial to man. Legal action in 1975 against the EPA led to the specification of 65 classes of toxic pollutants, the so-called consent decree priority pollutants which include the chlorinated hydrocarbon pesticides.

The EPA subsequently expanded the list of 65 classes to 129 specific pollutants. The priority pollutants also include the polychlorinated biphenyls (PCB's), a class of compounds used in electrical capacitors and transformers, plastics, paints and insecticides. The PCB's are very similar in properties to the chlorinated hydrocarbon pesticides. The properties of these compounds include toxicity towards various organisms, persistence in the environment, and response to analytical methods.

Several of the chlorinated hydrocarbon pesticides listed with the 129 priority pollutants have already been restricted by the EPA; they are aldrin, chlordane, DDD, DDT, dieldrin, endrin, heptachlor, and lindane (γ-BHC). The

others included on the priority pollutants list are the other three BHC isomers, heptachlor epoxide, DDE, the two endosulfan isomers, endrin aldehyde and toxaphene. The PCB's include seven major formulations, Aroclor 1016, 1221, 1232, 1242, 1248, 1254, and 1260. The last two digits indicate the percentage of chlorine present in the formulation which is a mixture of the polychlorinated isomers of biphenyl. The producer of the Aroclors, Monsanto Chemical Company, no longer manufactures these substances as they are being banned from the market effective 1 July 1979.

In order to meet the objectives of its mission, the EPA must have suitable analytical methods capable of determining the priority pollutants in air, water, and soil; the latter two media include many different mixtures or forms each of which can cause its own special analytical problems. To meet this need, the EPA sponsors programs aimed at the development, evaluation and testing of analytical methods for application in various aspects of environmental quality programs.

There are many ways in which the organic priority pollutants can enter the nation's waterways. The chlorinated hydrocarbon pesticides may be introduced through direct application to the land, from runoff from both domestic and urban areas, and by discharge from industrial operations. The PCB's enter the aquatic environment principally through the disposal of municipal and industrial wastes in dumps and landfills, although a significant amount may have been introduced as a contaminant in industrial wastewaters.

One of the main reasons for treating municipal wastewater has been to decrease the discharge of oxygen-demanding materials into the aquatic environment. Recently, this has been expanded to include the reduction of potentially toxic substances not only in the finished water discharged from these plants but also from the solid byproducts of municipal sewage treatment processes which are often used for landfill operations and, with slowly increasing frequency, as soil conditioners. The chlorinated hydrocarbons are both oxygen-demanding materials and known toxicants.

Since municipal wastewater contains not only domestic sewage but also storm water drainage, commercial wastes, and in some areas significant amounts of industrial wastes, the chlorinated substances can find their way into municipal treatment systems in large quantities. These substances do not usually have a marked deleterious effect on the treatment processes but can be returned to irrigation and drinking waters where they are toxic to various organisms, including the human populace. It then becomes imcumbent upon the authorities, in particular the EPA, to be able to monitor the presence of these materials and determine their fate and effects.

To remove pollutants a majority of munciipal sewage treatment facilities utilize the primary sedimentation process followed by biological activity to remove organics by contact with oxygen-activated microorganisms prior to disinfection and discharge. Analytical methods for the determination of chlorinated hydrocarbons in both the solid and liquid discharges from these processes are required to provide information as to their effects and final disposition.

This program was initiated by the EPA at Southwest Research Institute to develop and evaluate analytical methods to be applied to municipal sludges as a complement to on-going programs designed to provide analytical methods for water and wastewater. Specifically, this program was to provide a method for the qualitative and quantitative determination of the priority pollutant polychlorinated biphenyls and hydrocarbon pesticides in municipal sewage treatment plant sludges.

The analytical method was to include a means of removing the chlorinated substances from sludge and of detecting the individual compounds at a minimum concentration level of 300 ppb in the dry solids residue. The method also was to provide means of removing analytical interferences and of confirming the identity of any of the subject compounds detected.

The method developed as a result of this study includes extraction of the polychlorinated compounds by liquid-liquid partitioning, cleanup by removal of some interferences on a liquid chromatographic column and by precipitation of sulfur with mercury, concentration by evaporation of the extracting solvent, detection and quantification by electron capture gas chromatography, and confirmation of identity by gas chromatography/mass spectrometry.

SECTION 2

CONCLUSIONS

The results of experimental studies reported here have led to the formulation of an interim screening method for application to municipal sewage treatment plant sludges for compliance with the NPDES Permits Program. The method includes the following major steps:

1. extraction of the polychlorinated compounds from sludge by the centrifuge method, a liquid-liquid partitioning procedure,

2. isolation of the chlorinated compounds from some interferences by

   a. gel permeation chromatography to remove interfering organics, and
   b. removal of elemental sulfur by shaking with metallic mercury,

3. concentration of the chlorinated compounds to within the separation and detectability range of the determination method,

4. quantitative determination of polychlorinated compounds by gas chromatography/electron capture detection, and

5. confirmation of the identity of detected compounds by gas chromatography/mass spectrometry.

The minimum detectability limit attained is 0.3 µg of each single component pesticide per gram of dry sludge and 3-15 µg per gram dry sludge for the multicomponent polychlorinated formulations. These limits are based on observations of a small number of different sludges and may be subject to change as information is acquired from varying types of sludges.

The data collected on this program provide a basis for further experimentation to statistically characterize the reliability of the method, improve the various procedures, and to make further developments for qualification as a verification method. Refinement of the method will be required prior to its application to studies more rigorous than screening surveys.

SECTION 3

RECOMMENDATIONS

The various elements of this procedure should continue to be investigated. Whether this method meets the requirements of general applicability, statistical reliability, and economic considerations must be evaluated by the appropriate governmental agencies. The method provides a sound basis for the determination of the fate of the polychlorinated biphenyls and pesticides in municipal sewage treatment facilities.

Further development of the procedure is required in order to provide a more reliable method that provides better qualitative and quantitative analyses. Specifically, these studies should be continued and expanded to:

1. enhance the accuracy and precision of extraction techniques,

2. explore the modification of existing methods and/or use of other methods to enhance sample cleanup and remove more substances which interfere with determinative procedures.

3. develop the application of electronic integration and computer data handling methods to provide faster, more economical, and more accurate calculations,

4. investigate other gas chromatographic detectors which may enhance selectivity and obviate the need for more cleanup in the quantitative procedures.

5. compile mass spectral response data of reference compounds in sludge and form a data bank to make the method more applicable on a mass scale,

6. characterize sludges from facilities in different geographic areas which have varying influent loads from runoff, industrial, and domestic sources, and finally

7. integrate the method into an overall procedure for the determination of the 129 consent decree organic priority pollutants.

SECTION 4

EXPERIMENTAL PROCEDURES

GENERAL PROCEDURES

This program was initiated on a quick reaction basis in June 1978 to provide an interim method by December 1978 which could be used by the EPA for the NPDES Permits Program. Techniques and procedures were chosen for further study from those already available for water and wastewater after an assessment as to their stage of development and the probability that they could be successfully applied to municipal sludges. Consequently, most of the techniques and procedures were tested on a concurrent basis requiring a significant amount of duplication of effort. Electron capture gas chromatography was chosen as the quantitative step to be used throughout the program as it exhibits a high degree of slectivity and detection sensitivity for chlorinated hydrocarbons, has been the method of choice for many similar studies involving diverse sample matrices, and is fast becoming universally available in all types of analytical facilities, making it an economically attractive approach. Gas chromatography/mass spectrometry was chosen for qualitative confirmation of the chlorinated organic compounds.

The various elements of the experimental program conducted for this study were choice of sludge, determination of the solid residues, composition of the standard solutions, comparison of extraction and cleanup procedures, and application of analytical methods for determination and identity confirmation of the polychlorinated compounds.

CHOICE OF SLUDGE

Southwest Research Institute personnel have for several years collaborated with City of San Antonio officials on programs of several types, many of them of an environmental nature, and a good reciprocal working relationship has resulted. It was a simple matter, then, to make arrangements to obtain sludge from the city's Rilling Road sewage treatment plant on an as-needed basis.

Two types of sludge, primary and digested, were sampled by Institute personnel in 3-4 L batches and stored at 4°C in the sampling containers. The containers were wide-mouth glass jars which had been cleaned prior to sampling by detergent washing, solvent rinsing, and drying at 400°C. The caps were screwed on over a 0.02 mm Teflon film to prevent contamination from cap liner materials.

Later in the program, when it was desired to test the methodology with sludge from an industrial area, sludge samples were obtained from Dayton and Cincinnati, Ohio. Mr. D. F. Bishop of the Wastewater Research Division, MERL, USEPA made arrangements to have 3-4 L each of digested and combined sludge from Dayton and primary sludge from Cincinnati. The sludges were shipped under refrigeration to San Antonio where they were stored as before.

RESIDUE DETERMINATION

The method for determination of total residue was adapted from "Standard Methods for the Examination of Water and Wastewater" 14th Edition. It was necessary to continuously agitate a large amount of sludge while sampling in order to assure that a representative sample was taken.

SAMPLE AGITATION

In order to ensure homogeneity of samples throughout this study several ways of mixing the solids and liquids in sludge were investigated. Several devices were on hand and were used; these included a high-speed propellor-type paint mixer, a paint shaker, a Gifford-Wood industrial high-speed, high-shear homogenizer, and a hand-held stirring paddle. None of these methods was, by itself, very effective. Only the industrial homogenizer broke up all of the large pieces and resulted in a fairly uniform particle distribution. The problem with the Gifford-Wood instrument was that it whipped a great deal of air into the sludge making a froth and the solids stratified apparently as a function of their density. For example, a 3 L sample of San Antonio sludge taken from the primary clarifier was homogenized for one hour and samples were taken immediately from the top and bottom of the suspension. The average residue at the bottom was 0.8% total solids by weight for 5 determinations and at the top it was 3.2%. This stratification occurred regardless of the type of sludge used. A digested sludge agitated with the paint mixer resulted in a range of 6.6-9.4% total solids by weight.

A combination of techniques provided the best results. The sludge was homogenized with the Gifford-Wood unit, allowed to set undisturbed for the entrained air to escape, and then agitated with a propellor-type stirrer while sampling. This was an effective procedure as long as the stirring was done carefully; i.e., the solids were kept evenly distributed but no air was whipped into the suspension.

Determination of total solids in the San Antonio sludges resulted in the following values:

$$\text{Primary sludge} = 3.59 \pm 0.06\%$$
$$\text{Digested sludge} = 3.81 \pm 0.05\%$$

The Dayton and Cincinnati sludges were used to compare total solids determinations from sludges which were sampled while stirring only or while being stirred after homogenization. The results of these determinations are shown as Table 1. There are apparently no differences between the two values for each type of sludge. An analysis of variance on the experimental

data indicated this was in fact correct. The main source of error expected to arise would be from any large pieces of material in the sludge which would not be broken up by a propellor stirrer and hence, would not get into an analytical procedure. For this reason, all sludge samples were homogenized with the Gifford-Wood instrument prior to any other operations.

GAS CHROMATOGRAPHIC DETERMINATIONS

The gas chromatographic determinations made in this study were all done with the same system. The gas chromatograph was equipped with a 1.8 m long x 4 mm I.D. glass column packed with 1.5% SP-2250/1.95% SP-2401 on 100/120 mesh Supelcoport.[R] The helium carrier gas flowed at a rate of 60 mL/min through the column which was maintained at 200°C. Injection was on column at 225°C and detection was by linearized electron capture at 300°C. Electron capture is highly sensitive to polychlorinated compounds such as those included on the priority pollutants list and studied here.

The individual compounds may be detected in amounts of a few tens of picograms (10^{-12}g). The required minimum detectability of 300 ng in 1 g of solids could then be achieved by extracting 20g of a 5 percent solids sludge, concentrating the extract to 10 mL and injecting 10 µL which would contain 300 ng of any compound present at the 300 ppb level in the solids. This would be adequate for any single compound but it would be quite difficult to detect the multicomponent formulations, chlordane, toxaphene, and the Aroclors.

Information from the wastewater phase of this contract dealing with the polychlorinated compounds indicated that minimum detectability expected for chlordane would be about 3 µg in 1 g of solids under the conditions stated above. Minimum detectability expected for toxaphene would be about 15 µg in 1 g solids and for AR 1260, the PCB chosen for these studies, about 6 µg in 1 g.

Authentic reference standards of the 17 formulations were obtained from the EPA Health Effects Research Laboratory at Research Triangle Park, North Carolina. These compounds were dissolved singly and in mixtures at appropriate concentrations and their gas chromatographic retention times were determined. Retention times relative to aldrin were calculated and are shown in Table 2.

The 17 chlorinated organic compound formulations were separated into five groups for convenience of handling. Group I was made up of α-, β-, and δ-BHC, p,p'-DDE, p,p-DDD, p,p-DDT and heptachlor epoxide; the chromatogram of this mixture of standards is shown as Figure 1. Group II included the compounds γ-BHC, aldrin, endosulfan I and II, dieldrin, endrin and its aldehyde, and heptachlor. The chromatogram of the Group II mixture of standards is shown as Figure 2. The multi-component formulation, chlordane, makes up Group III, and its chromatogram is shown as Figure 3. Group IV is made up of the many compounds which together constitute the pesticide, toxaphene. The Group IV chromatogram is shown as Figure 4. It is apparent from these chromatograms that the multi-component pesticides are quite complex and not always easy to recognize. Batch to batch variations of the

TABLE 1. COMPARISON OF METHODS FOR HOMOGENEOUS SAMPLING OF SLUDGE

Type of Sludge	Mixing Method	Total Solids*
Dayton Combined	Stirred† by propellor	6.26 ± 0.00
Dayton Combined	Homogenized‡ and stirred	6.36 ± 0.07
Dayton Digested	Stirred by propellor	5.90 ± 0.02
Dayton Digested	Homogenized and stirred	5.85 ± 0.01
Cincinnati Combined	Stirred by propellor	6.23 ± 0.03
Cincinnati Combined	Homogenized and stirred	6.19 ± 0.06

* Mean of three replicate determinations ± standard deviation

† Stirred during sampling; no prior treatment.

‡ Homogenized and allowed to deaerate prior to sampling; stirred during sampling.

TABLE 2. RELATIVE RETENTION TIMES OF POLYCHLORINATED PESTICIDES AND AR 1260

Compound	RRT*
α-BHC	0.56
γ-BHC	0.69
β-BHC	0.78
Heptachlor	0.84
Chlordane† (C-2)	0.84
δ-BHC	0.90
Aldrin	1.00
C-3	1.13
Heptachlor Epoxide	1.46
C-4	1.62
C-5	1.75
Endosulfan I	1.82
DDE	2.12
Toxaphene† (T-1)	2.20
Dieldrin ‡	2.21
Aroclor 1260† (A-1)	2.38
A-2	2.63
Endrin	2.66
A-3	2.98
C-6	3.12
DDD	3.20
Endosulfan II	3.23
T-2	3.28
A-4	3.36
A-5	3.79
DDT	3.86
T-4	3.97
Endrin Aldehyde	5.95
A-6	5.95

* Calculated relative to Aldrin
† Multicomponent pesticide formulation.
‡ Multicomponent PCB formulation.

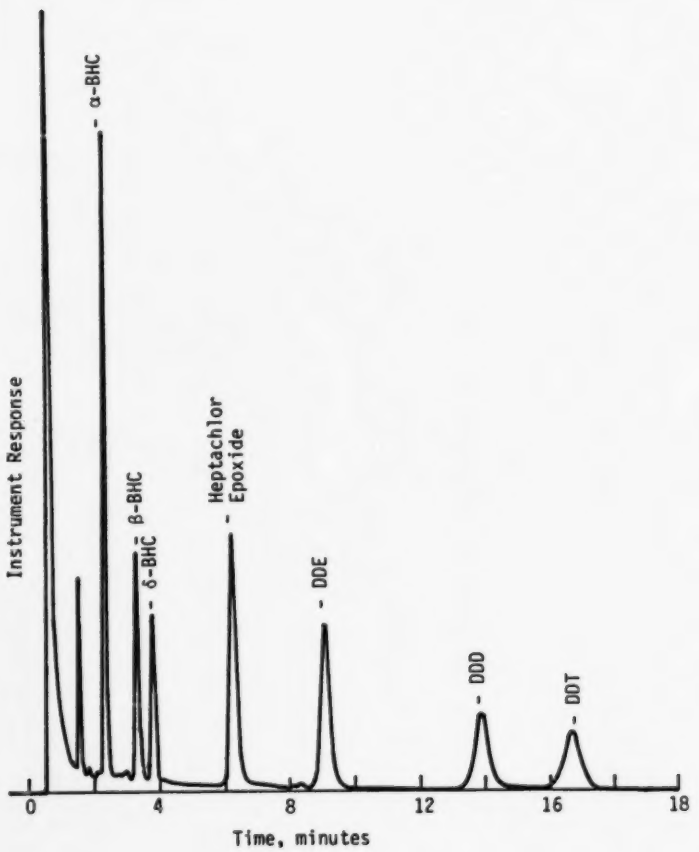

Figure 1. Electron capture gas chromatogram of Group I reference standards.

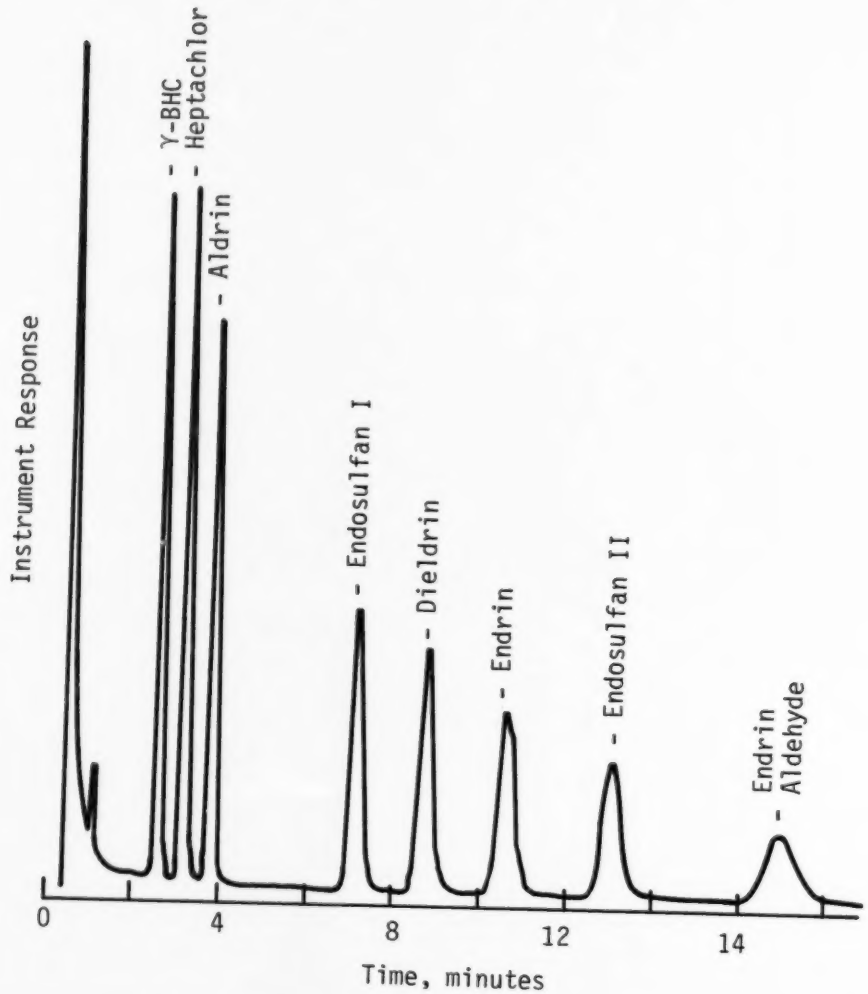

Figure 2. Electron capture gas chromatogram of Group II reference standards.

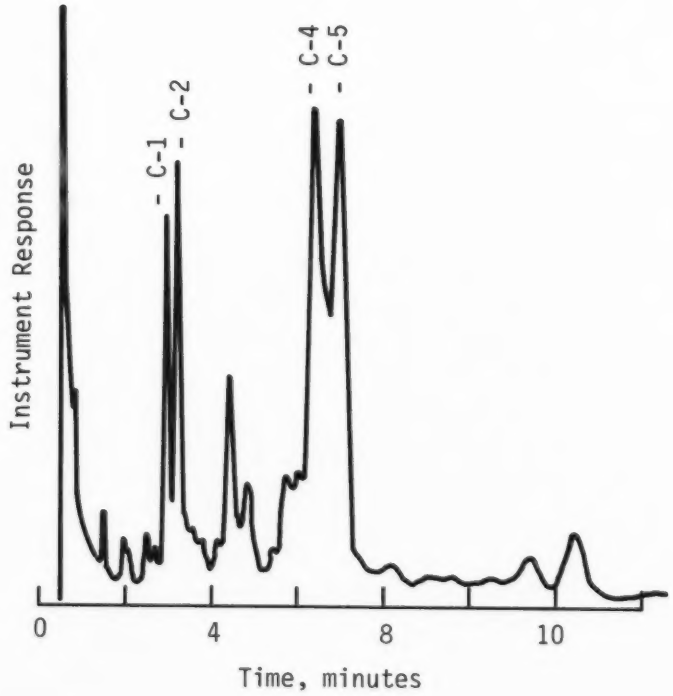

Figure 3. Electron capture gas chromatogram of Group III, chlordane, reference standard.

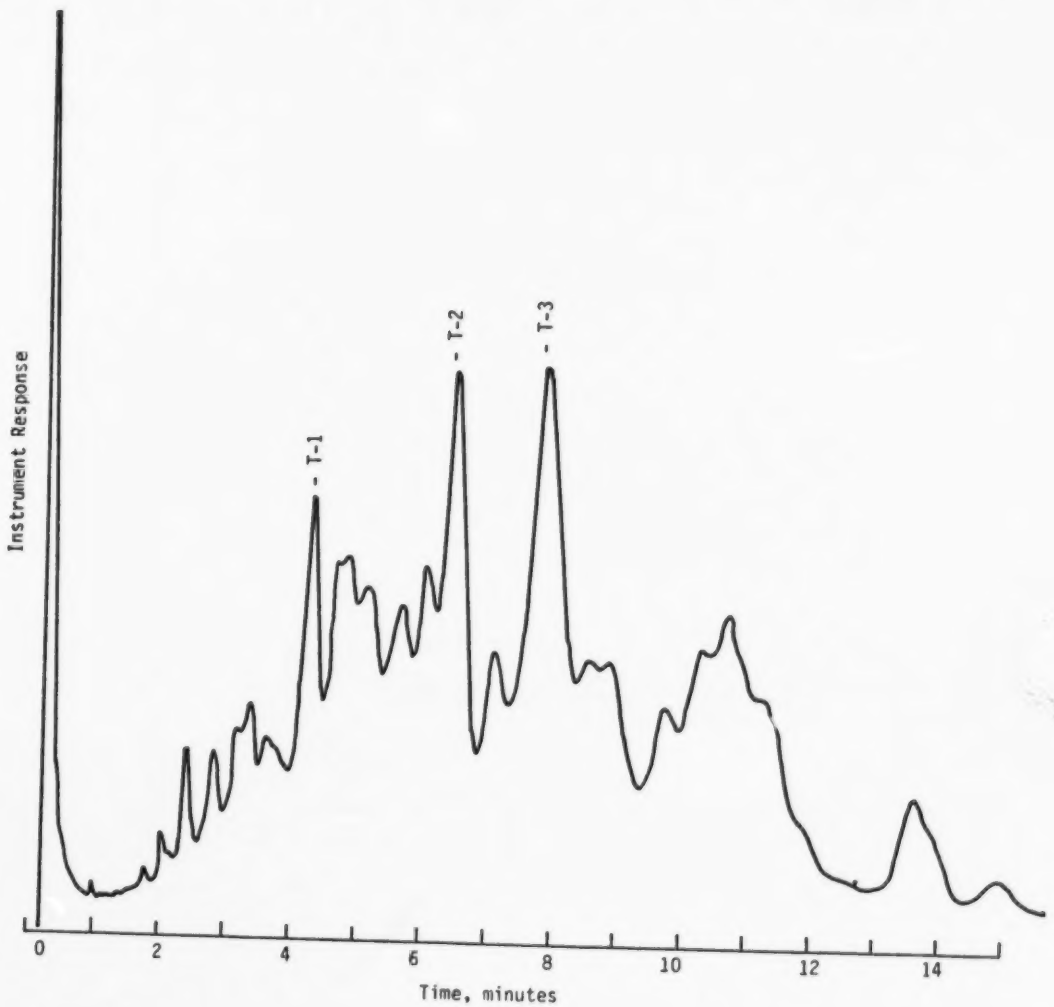

Figure 4. Electron capture gas chromatogram of Group IV, toxaphene, reference standard.

relative amounts of the compounds in these formulations can also serve to complicate their analytical recognition as can any compounds that elute near to those of either chlordane or toxaphene. An analyst attempting to determine these compounds in a complex matrix such as municipal sludge must be thoroughly familiar with the response of toxaphene and chlordane under a variety of conditions and be able to recognize them when they are apparently not present. Access to gas chromatography/mass spectrometric instrumentation makes the identification of these mixtures much less doubtful.

The final mixture is Group V, Aroclor 1260, a formulation of a number of polychlorinated biphenyls which together have a chlorine content of 60 percent indicating a high degree of chlorine substitution. The gas chromatogram of Group V is shown as Figure 5. The PCB's are made up of compounds which are very similar to those of the pesticides. For this reason the PCB's and pesticides are mutual interferences and it requires a highly experienced analyst to be able to recognize one in the presence of the other. The PCB's, as the pesticides, are subject to interference from other electron-capture-responsive substances found in the sample matrix. Although Aroclor 1254 was one of the most used PCB's, we chose to work with Aroclor 1260 for this study because it includes most of the compounds that 1254 has and enough of the more highly chlorinated ones to extend the gas chromatographic region beyond that of the pesticides.

EXTRACTION METHODS

The extraction procedures in an analytical method can often be the most critical. The ideal extraction would remove only the analyte compounds and without any additional manipulations provide them in the concentration necessary for analytical determinations. This of course is seldom the case. Municipal sludges, because of the complexity of compounds present, present an extreme deviation from the ideal. The nature of these compounds in the sludge presents a certain advantage to extraction by partitioning into a water-immiscible organic solvent. Many of these compounds are of a fatty, oily, or waxy nature and would be expected to selectively dissolve compounds such as the polychlorinated hydrocarbons. This mixture would then be present as a distinct phase in the system, whether separated from the water or suspended in it, and should partition readily into an extracting organic solvent.

With this in mind four extraction methods were chosen for evaluation, 1) liquid-liquid partitioning between immiscible solvents, 2) percolation of an organic solvent over sludge which had been rendered granular and free flowing by the addition of a salt having a high capacity for taking up water, 3) Soxhlet extraction of sludge granulated as in 2, and 4) continuous liquid-liquid extraction in a modified soxhlet apparatus. The latter two methods require continuous heat input and raise the question of the possibility of reaction or degradation of the analytes. This was not a major concern since continuous extractions under reflux have been used successfully for the extraction of polychlorinated compounds in other systems.

Liquid-liquid partitioning was first tested by shaking a 20 g sample of sludge with dichloromethane (DCM) in a separatory funnel. An emulsion formed

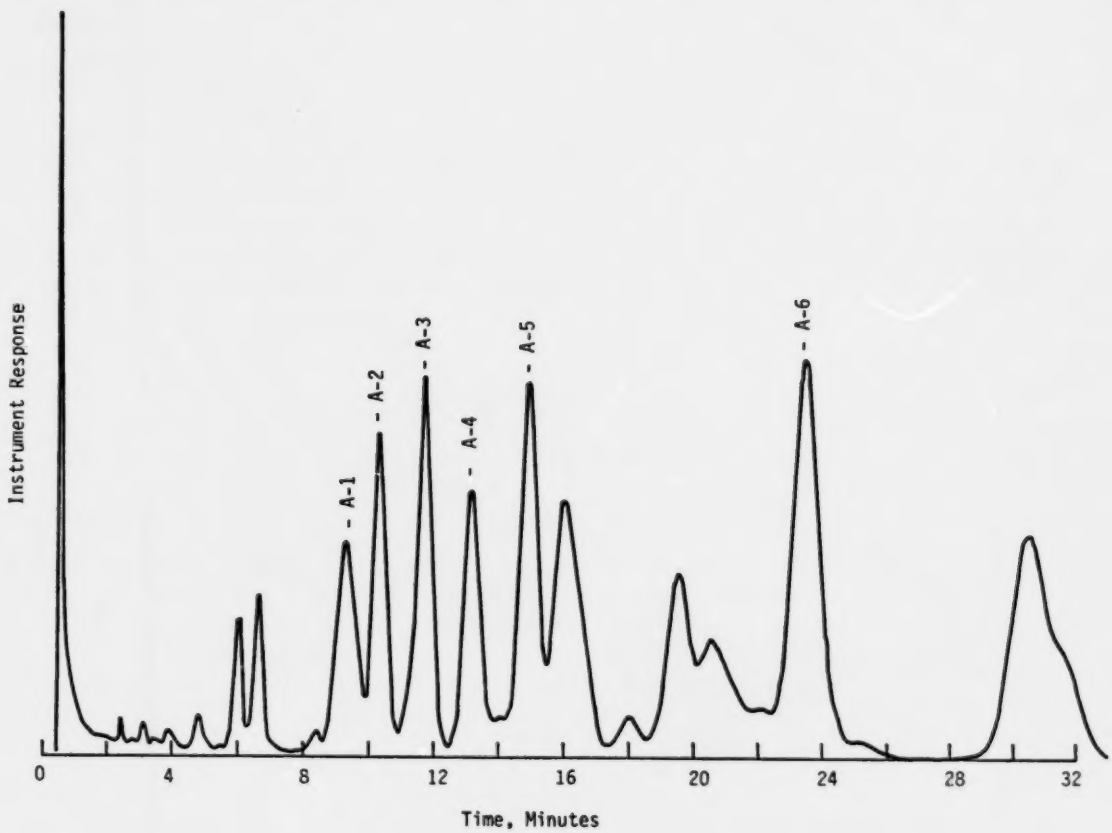

Figure 5. Electron capture gas chromatogram of Group V, Aroclor 1260, reference standard.

which was extremely difficult to break even when the ratio of solvent to sludge was increased to 25:1. This method held little promise unless some means of breaking the emulsion and separating the liquid phases could be provided.

Centrifugation is a convenient means of breaking emulsions and the necessary equipment is generally available in most laboratories. It was found that centrifugation would effectively break an emulsion formed by sludge with either DCM or hexane. The DCM, since it is more dense than water, settled to the bottom of the centrifuge tube and had to be removed by inserting a pipet, large syringe needle, or aspirator tube through the aqueous and solids layers and usually a small amount of the sample matrix was withdrawn the solvent.

It was decided to use a solvent mixture of hexane, DCM, and acetone for further experimentation with the "centrifuge method" of extraction. The hexane, in addition to being a solvent for the polychlorinated compounds, would act as the vehicle for the extracting solvent, DCM would enhance the solvation properties, and acetone would promote invasion of the interstices of the solid matrix.

The stepwise procedure for evaluation of the centrifuge method of extraction follows:

1. Weigh a 20 g sludge sample into a 200 mL Pyrex screw-cap centrifuge bottle.
2. Add 20 mL H_2O saturated with NaCl.
3. Add 60 mL hexane-DCM-acetone (83:15:2) and seal the bottle with a teflon-lined cap.
4. Shake the bottle vigorously for at least 1 min. by hand or with a mechanical homogenizer such as with Tekmar Tissuemizer® or Brinkman Polytron.®
5. For phase separation, centrifuge at 500XG for 20 min. at 15°C.
6. Carefully pipet or aspirate the upper solvent layer and transfer to a suitable receiving vessel.
7. Repeat steps 3-6 twice with 60 mL volumes of extracting solvent.
8. Combine the solvent extracts and retain for further procedures.

The column elution extraction method was adapted from methods used in these laboratories for the extraction of pesticides and PCB's from soils and sediments. The extracting solvent had to contain a water-miscible solvent such as acetone in order to provide intimate contact with the water occluded within the pores of the solid matrix. A mixture of 20% acetone in hexane was chosen for evaluation of the column elution method of which stepwise details follow.

1. Weigh a 20 g sludge sample into a beaker

2. Add 80 g anhydrous, granular Na_2SO_4 and mix well until the mixture is free flowing

3. Transfer the granular mixture to a 20-mm diameter glass column having a glass wool plug at the bottom

4. Wash the beaker with the extracting solvent, 20% acetone in hexane, into the column continuing until a total of 150-mL solvent has been added

5. Collect the eluate in a suitable vessel and retain for analytical determinations.

Extraction of granulated sludge with a Soxhlet apparatus is based on principles similar to those of the column elution method with two differences in the methodology. The extraction can be continued over long periods of time since the solvent can be continuously recycled by refluxive distillation through a sample matrix, ostensibly enhancing solvation of the analytes. Also, a water-miscible solvent is not used as it would carry over a large volume of water into the extract because of the continuous cycling action of the technique. The procedural details of the Soxhlet extraction method follow:

1. Weigh a 20 g sample of sludge into a beaker.

2. Add 180 g anhydrous, granular Na_2SO_4 and stir until the mixture is free flowing.

3. Transfer the granulated sludge to a glass extraction thimble having a plug of glass wool on the frit and place another plug over the sample.

4. Charge the extractor flask with 200 mL 15% DCM in hexane which has been used to wash the beaker which contained the sludge.

5. Activate the heat controls and run for four hours at a cycle rate of 8-10/hour.

6. Retain the extract for further analytical procedures.

The continuous liquid extraction method began with a handicap that could not be overcome. The method requires that the extracting vessel provide some means of mixing the sample and extracting phases on a continuous basis. This type of apparatus was not available in this laboratory and could not be obtained on a timely basis. Several Soxhlet apparatuses were modified to return the extracting solvent to the still-pot without siphoning by raising the siphon tube connection on the return tube. In this manner, extracting solvent would flow continuously through the return tube as it condensed and dripped through the sludge. The procedural details of this method are:

1. Place a plug of glass wool in the return tube where it connects to the extractor body (this will keep entrained solids from flowing into the distilling flask).

2. Place 175 mL DCM in the body of a modified Soxhlet extractor collecting any overflow in the distilling flask.

3. Weigh 20 g sludge into a small beaker.

4. Transfer the sludge to the extractor washing 3 times with ≈10 mL water and finally with ≈10 mL DCM.

5. Turn on the heat to the still-pot and extract ≈16 hours (overnight).

6. Retain the extract for further analytical procedures.

These procedures were then evaluated for extraction efficiency by spiking sludge with known quantities of authentic standards, extracting by the listed methods, removing interferences and analyzing by the procedures which follow. Also, the effect on extraction efficiency of shaking or blending the sludge/solvent mixture in the centrifuge method was determined.

CONCENTRATION AND CLEANUP

The sample extracts require concentration prior to other operations such as cleanup or analysis. Concentration is normally achieved simply by evaporating part of the extracting solvent, usually in a Kuderna-Danish apparatus on a steam bath. Concentration to volumes less than 5-8 mL cannot be carried out effectively on a macro Kuderna-Danish apparatus. This type of concentration can be carried out by directing a stream of dry, inert gas onto the extract solution and evaporating the solvent to the desired volume. An alternate method of concentrating to small volumes is the use of a micro Kuderna-Danish apparatus although caution must be exercised as this method can result in significant analyte losses. Evaporation to volumes less than 5 mL is avoided as it often involves losses of the analyte compounds. It is required for such operations as gel permeation chromatography and great care needs to be exercised especially to prevent the solution from going to dryness.

The chlorinated solvent, DCM, used in most of the solvation/extraction operations interferes with use of the electron capture detector. A large amount of DCM causes a detector response which interferes with the determination of early eluting chlorinated compounds. DCM is removed from the solution simply by adding 10-25 mL hexane to the 6-8 mL concentrate in the Kuderna-Danish apparatus and evaporating again on the steam bath.

Interferences are usually removed from the concentrated extracts prior to gas chromatographic analysis. Two methods for the removal of organic compounds and three for the removal of elemental sulfur were tested. Organic interferences were removed by liquid chromatographic fractionation on

Florisil and by steric exclusion chromatography on Sephadex LH-20 or Bio-Beads S-X2. The removal of elemental sulfur was studied by contact with activated copper metal or elemental mercury and by conversion to thiosulfate for removal by dissolution in water/alcohol.

Florisil, which has been activated at 675°C, is heated at 600°C for two hours prior to use. Five percent by weight water is added to the Florisil after cooling and allowed to equilibrate for 24 hours while periodically shaking vigorously. Twenty g of the H_2O-deactivated Florisil is charged in a 20 mm diameter glass column and is settled by tapping the column gently. A 10-15 mm layer of anhydrous, granular Na_2SO_4 is added and the column is washed with 75 mL hexane which is discarded. The sample extract is quantitatively transferred in 10 mL hexane to the column and the solution allowed to just disappear at the bottom of the sulfate layer.

The first fraction is eluted with 150 mL 6% diethyl ether in hexane and the second with 150 mL 50% ether/hexane. The fractions are caught in a Kuderna-Danish flask in preparation for evaporation of the solvent prior to other operations of the analytical protocol being conducted. All of the polychlorinated priority pollutants elute in the 6% ether fraction except for endosulfan II and endrin aldehyde which appear in the 50% ether fraction.

Both Sephadex LH-20 and Bio-Beads S-X2 were evaluated for removal of high molecular weight interfering materials from the sludge extracts. After original work showed the LH-20 to exhibit some adsorption in addition to steric exclusion properties, the Bio-Beads S-X2 was used exclusively. The following procedure was identical for either gel material.

To prepare the gel material for use, weigh 25 g into a beaker, add 2-3 volumes of cyclohexane, cover and let stand overnight. Transfer the swollen gel to a 2 mm diameter glass column equipped with a stopcock and allow to settle to a bed volume of approximately 50 cc. Flush the column with 75-100 mL cyclohexane under an inert gas pressure of ≈62 kPa being careful not to let the gel go dry. Allow the cyclohexane to drain flush with the top of the gel bed and transfer 8 mL of the sludge extract to the top of the gel bed. Wash the sample vial and sides of the column 3-4 times with 2-4 mL of cyclohexane and allow the solvent to drain to the top of the gel bed. Fill the column with cyclohexane and elute at 5 mL/min under an inert gas pressure of ≈62 kPa and collect six 25 mL fractions in Kuderna-Danish concentrators. Evaporate each fraction to a volume less than 5 mL, transfer in small portions to a 0.5 mL screw cap vial and maintain a stream of pure, dry inert gas on the solution until all has been transferred and it has been evaporated to ≈0.1 mL. Seal with a metal foil- or Teflon-lined cap and store at 4°C.

This cleanup method was used for the GC/MS confirmation of identity of compounds which had been determined by GC/ECD. Another method was developed with Bio-Beads S-X2 gel which provides a fairly sharp separation between high molecular weight compounds which are discarded and polychlorinated compounds which elute in a relatively small volume.

The S-X2 gel is prepared for use by weighing 30-40 g into a beaker, adding 2-3 volumes of DCM, and allowing to stand sealed overnight. Then transfer the swollen gel in portions to a 22 mm diameter, 400 mm long glass column equipped with a Teflon stopcock plug and allow it to settle until a stable bed height of 300-310 mm is achieved. Allow the DCM to drain flush with the top of the bed being careful not to let any part of the gel go dry. Place a 15-20 mm layer of anhydrous, granular sodium sulfate and add enough DCM to cover it.

The sludge extract is concentrated by Kuderna-Danish evaporation to 5-10 mL, 75 mL DCM is added and the evaporation is repeated to a final volume of 2-3 mL. The body of the concentrator is washed with a small volume of DCM into the collection tube and final concentration is done by placing the tube in a beaker of warm water (35°C-40°C) and directing a stream of dry, inert gas onto the solution until a final volume of 2-3 mL is achieved.

Carefully drain the excess DCM in the gel column into the Na_2SO_4 until just above the gel bed and close the stopcock. Transfer the sludge extract to the top of the gel column and allow to drain to the top of the gel bed. Rinse the sample tube with 2-3 small DCM volumes and pour into the column; the sample volume plus the rinsings should not exceed 5 mL. Allow the DCM to elute to the top of the gel layer, add 1-2 mL DCM and repeat. Add DCM to the column, begin the elution, and maintain the DCM at a level 20-40 mm above the Na_2SO_4.

Under these conditions the DCM will flow at a rate of approximately 3 mL/min. Collect the first 90 mL of eluate, which contains a large part of any inteferences, and discard. Collect the next 25 mL containing the polychlorinated compounds, seal the container and store refrigerated.

CONFIRMATION OF IDENTITY

Extracts of municipal sewage treatment sludge are normally quite complex and will contain a large number of compounds that cause a response of the electron capture detector. Consequently, a positive response at a point corresponding to the elution of one of the compounds listed in Table 2 cannot be considered absolute proof that such a substance is present. Gas chromatography/mass spectrometry provides an ideal means for confirmatory identification of those polychlorinated compounds presumed to be in municipal sludge extracts from gas chromatographic/electron capture detector data.

The GC/MS determinations were made on the single extract from the S-X2 DCM clean-up and alternatively, when required, on the six fractions of sludge extract collected from S-X2/cyclohexane chromatography. The instrument used was a U-column gas chromatograph interfaced to an electron impact, quadrupole filter mass spectrometer. Data were recorded and analyzed by a computer data system. The instrument is manufactured by the Finnigan Corporation, Sunnyvale, California.

Approximately 3 µL of concentrated extract fraction of sludge was injected for each gas chromatographic run. The chromatographic conditions were set to duplicate the results of the electron capture determinations.

The same column material was used in a 1.6 m long, 2 mm diameter column. The overall procedure was followed as closely as possible to that from the EPA manual, "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants," April 1977.

SECTION 5

EXPERIMENTAL RESULTS

GENERAL PROCEDURES

The first experiments conducted were the comparisons of extraction methods on sludges from the San Antonio Rilling Road Sewage Treatment Plant spiked with known amounts of polychlorinated standards. One extraction method was chosen and two methods of chromatographic cleanup were compared, while three methods of removing elemental sulfur were compared. All of the quantitative determinations were done by electron capture gas chromatography. When all of the individual parts of the analytical procedure were chosen, gas chromatography/mass spectrometry was investigated for the confirmation of identities. The integrated method was also tested for its applicability to sludges from plants in Dayton and Cincinnati, Ohio which were expected to have a larger contribution of industrial wastes than the San Antonio sludge.

EXTRACTION METHODS

The results of extraction of San Antonio sludges spiked with known amounts of polychlorinated compounds are shown in Tables 3 and 4. Sample weight was 20g and the sample spiked with Group I or Group II contained 1 µg of each compound, Group III 10 µg of chlordane, Group IV 50 µg of Toxaphene, and Group V 20 µg of Aroclor 1260. These recovery values were calculated from peak areas recorded by digital integration techniques with a laboratory computer system interfaced to the gas chromatograph through an electronic analog-to-digital converter. The chromatograms were complex and caused a greater scatter in calculated peak areas because the computer could not recognize and differentiate among peaks. Very slight differences in peak characteristics from one run to the next could cause the computer to lose or gain one to three peaks in a peak area measurement.

The chromatograms shown in Figure 6 may be used to point out the problems in computing peak areas. The peak area calculated for α-BHC in the spiked sludge would include that of the peak immediately preceding it in the unspiked sludge. Similarly the peak area for DDD in the spiked sludge would include that of the peak which immediately follows it and shows up as only a faint shoulder. The peak area of DDT may or may not contain the areas of the peak trailing after it depending on whether the slope sensitivity of the integrator would recognize the slight dip as an inflection point. These changes in several cases are subtle enough that the chromatograms must be visually inspected to recognize them.

TABLE 3. COMPARISON OF EXTRACTION TECHNIQUES FOR SINGLE COMPONENT PESTICIDES

		Percent Recovery*													
		Group I							Group II						
Method	Sludge Type+	α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT	γ-BHC	Hept.	Aldr.	End. I.	Diel.	Endr.	End. II	Endr. Ald.
Centrifuge	P	84	80	66	95	81	82	59	90	87	84	98	76	78	96	12
	D	171	148	122	96	188	60	2	66	79	98	89	129	138	88	14
Column Elution	P	102	86	75	109	103	102	79	80	99	95	106	98	72	116	7
	D	216	198	134	86	166	76	15	141	111	169	93	173	147	91	9
Soxhlet	P	95	100	70	97	86	91	70	85	94	72	106	89	73	114	14
	D	189	316	199	168	370	107	58	110	146	119	56	100	91	61	12
Liquid-Liquid	P	54	62	38	47	37	37	35	134	78	86	90	80	75	87	2
	D	4	0	0	0	2	0	0	54	13	16	17	10	18	18	4

* Average of two determinations for each sludge type and method except liquid-liquid only one determination was made for each sludge type; cleanup by Florisil column chromatography.

\+ San Antonio sewage treatment plant; P = primary sludge, D = digested sludge.

TABLE 4. COMPARISON OF EXTRACTION TECHNIQUES FOR MULTICOMPONENT FORMULATIONS

Method	Sludge † Type	Percent Recovery *													
		Group III- Chlordane					Group IV-Toxaphene			Group V - Aroclor 1260					
		C-2	C-3	C-4	C-5	C-6	T-1	T-2	T-4	A-1	A-2	A-3	A-4	A-5	A-6
Centrifuge	P	93	96	60	80	30	120	79	115	91	100	103	98	104	90
	D	75	50	81	86	56	102	90	92	58	78	82	76	78	108
Column Elution	P	44	66	99	98	44	148	123	118	86	117	123	117	104	126
	D	90	92	72	74	54	114	92	119	118	129	129	128	132	137
Soxhlet	P	72	10	76	87	59	131	98	98	69	89	94	93	96	110
	D	98	107	107	119	82	114	89	109	77	94	96	95	94	100
Liquid-Liquid	P	44	29	34	43	45	71	63	59	14	19	19	16	18	18
	D	1	5	14	14	7	31	19	19	56	61	60	59	63	64

* Average of two determinations for each sludge type and method except liquid-liquid; cleanup by Florisil column chromatography.

† San Antonio sewage treatment plant; P = primary sludge, D = digested sludge.

It was felt that manual measurement of peak heights would provide more reliable calculations. Consequently, all subsequent calculations were based on peak height measurements made manually; i.e., by hand and rule.

The results shown in Tables 3 and 4 on first glance would only rule out the liquid-liquid method on the basis of very low recoveries. But closer evaluation of the data in combination with the visual evaluation of the more than 300 chromatograms these data were taken from led us to choose the centrifuge method of extraction for further study. Other considerations such as the possibility of thermally induced reactions in the Soxhlet and liquid-liquid methods, the apparently greater variability of results from the column elution method, the limited availability and greater expense of continuous liquid-liquid extractors, and the assessment of laboratory personnel that they had a greater degree of control over the manipulations of the centrifuge method served to reinforce this decision.

Another set of experiments was conducted with San Antonio primary sludge to further test the centrifuge method. Each of the five groups of compounds was spiked into individual 20g wet sludge samples, equilibrated for 18 hours, and extracted by the centrifuge method. After cleanup the extracts were chromatographed and recoveries of the individual compounds were calculated from both computer integration and peak height data. Each group determination was accompanied by extraction of unspiked sludge replicates to provide correction of the data by subtraction of "blank" values. The data obtained from these experiments are shown in Tables 5 and 6 as average percent recovery for each compound.

The peak height data exhibit more reasonable recovery values especially for Groups I and II. There was good precision between replicates for both the computer integration and peak height measurements but the latter was more reliable because the analyst could adjust to chromatographic anomalies whereas this was not always possible with computer integration even by changing the calculation program parameters.

Recoveries were fairly consistent by the peak height calculations, in the 70 to 90% range for the most of the Group I and II compounds at the higher concentration levels. Recoveries were noticeably less consistent for the lower spike levels, especially for the Group II compounds. Groups III, IV and V, the multicomponent formulations, exhibited very inconsistent recoveries for the compounds within the group. For example, recoveries of the five peaks calculated for chlordane ranged from 25% to 90% by the peak height method and 44 to 92% by computer integration.

At this time, based on these results, the decision was made to make all subsequent calculations of recoveries by the peak height method. Although more tedious and time consuming, it was felt that more reliable data would result for the determination of procedural efficiences.

The centrifuge extraction method was then applied to sludges obtained from Cincinnati and Dayton, Ohio municipal sewage treatment plant facilities. As with the San Antonio sludges, 20g aliquots were spiked at two levels,

TABLE 5. COMPARISON OF AREA INTEGRATION AND PEAK HEIGHT CALCULATIONS, GROUPS I & II

Spiking Level µg/l *	Calculation Method †	Average % Recovery of Single-Compound Pesticides													
		Group I							Group II						
		α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT	γ-BHC	Hept.	Aldr.	End.I	Diel.	Endr.	End.II	Endr. Ald.
15	CI	86	104	89	97	104	92	58	79	76	82	90	104	98	98	26
	PH	94	90	79	83	77	78	54	100	86	79	92	94	89	94	28
50	CI	68	128	97	104	98	97	55	28	30	37	87	89	52	82	34
	PH	98	99	78	84	71	84	55	89	76	63	140	85	62	92	45

* Wet sludge; cleanup by Florisil column chromatography.

† CI = computer integration of area; PH = peak height measurement.

TABLE 6. COMPARISON OF AREA INTEGRATION AND PEAK HEIGHT CALCULATIONS, GROUPS III-V

Spiking Level µg/l*	Calculation Method †	Average % Recovery of Multicomponent Pesticides and Aroclor												
		Group III-Chlordane					Group IV-Toxaphene			GROUP V - AR 1260				
		C-2	C-3	C-4	C-5	C-6	T-1	T-2	T-4	A-2	A-3	A-4	A-5	A-6
150	CI	88	51	75	75	48								
	PH	80	60	80	68	25								
500	CI	92	44	85	80	54								
	PH	80	76	88	90	53								
750	CI													
	PH						206	258	156					
							125	113	87					
2500	CI													
	PH						68	98	122					
							96	90	84					
300	CI													
	PH									100	90	98	76	84
										102	76	101	92	106
1000	CI													
	PH									70	74	75	71	77
										89	80	86	86	91

* Wet sludge; cleanup by Florisil column chromatography.

† CI = computer integration of area; PH = peak height measurement.

generally 100% and 30% of a nominal level in 20g wet sludge. The nominal (100%) level was 1 µg for Groups I and II, 10 µg for Group III, 50 µg for Group IV and 20 µg for Group V. This provides spike levels of 1 and 0.3 µg, 10 and 3 µg, 50 and 15 µg, and 20 and 6 µg, respectively. For the sake of consistency and simplicity, spiking was done on a wet sludge basis. This normally made little difference since solids content in all of the sludges used was fairly consistent within the range of 3.5-6%. These levels were used for all determinations from this point on and will simply be referred to as the 100% and 30% spike level.

The sludges were extracted by the centrifuge method, cleaned up and analyzed in a manner identical to the preceding work. Representative chromatograms of the three sludges, both spiked and unspiked are shown as Figures 6-9. These chromatograms show strong evidence for the presence of the Group III pesticide, chlordane. The four peaks normally measured for chlordane are quite apparent in the unspiked sludges in the same relative amounts as the authentic standards. These sludges also exhibited responses for the BHC's, both endosulfans, DDT, heptachlor epoxide, and Aroclor 1260.

Electron capture chromatography of all sludges demonstrated about the same background levels. Perhaps the San Antonio sludge showed slightly lower background levels than the other possibly due, in part, to a smaller percentage of wastewater influent from industrial sources. In all types of sludges, difficulty was encountered in trying to use computer integration for determination of peak areas which necessitated peak height measurement for all the data obtained. The percent recoveries obtained using the three sludges from different locations are summarized in Tables 7 and 8.

For Group 1, the recoveries were reasonably close to those from the San Antonio sludge for α-BHC and DDE. The percent recovery of α-BHC was high for all sludges, indicating at least 80 percent can be expected down to the 0.3 µg/20 g level, and recoveries above 90 percent occurred frequently. The results for the combined sludge were slightly lower for DDE, but were acceptable at the higher spike level. The recovery of DDT from the sludges studied here was significantly higher than obtained on the San Antonio sludge in most cases, and recovery of heptachlor epoxide was roughly equivalent. The remaining compounds gave quite variable results depending upon the spike level and the matrix.

For Group 2, the most significant result was the poor results obtained using the low level spike on the primary sludge. Only four of the eight compounds were recovered at all, and these were quite low. In general, much better recoveries were observed for the 1.0 spike vs the 0.3 spike, similar to those seen in the San Antonio sludge. Endrin aldehyde was only infrequently recovered in the three sludges, compared with the 45-47 percent recovery obtained from the San Antonio sludge.

Recoveries for the 1.0 µg/20g spike were acceptable, in general, with Endosulfan I being the principal exception. At the 1.0 spike level, highest recovery was 58.7 percent for the combined sludge, and ranged to 31.4 percent

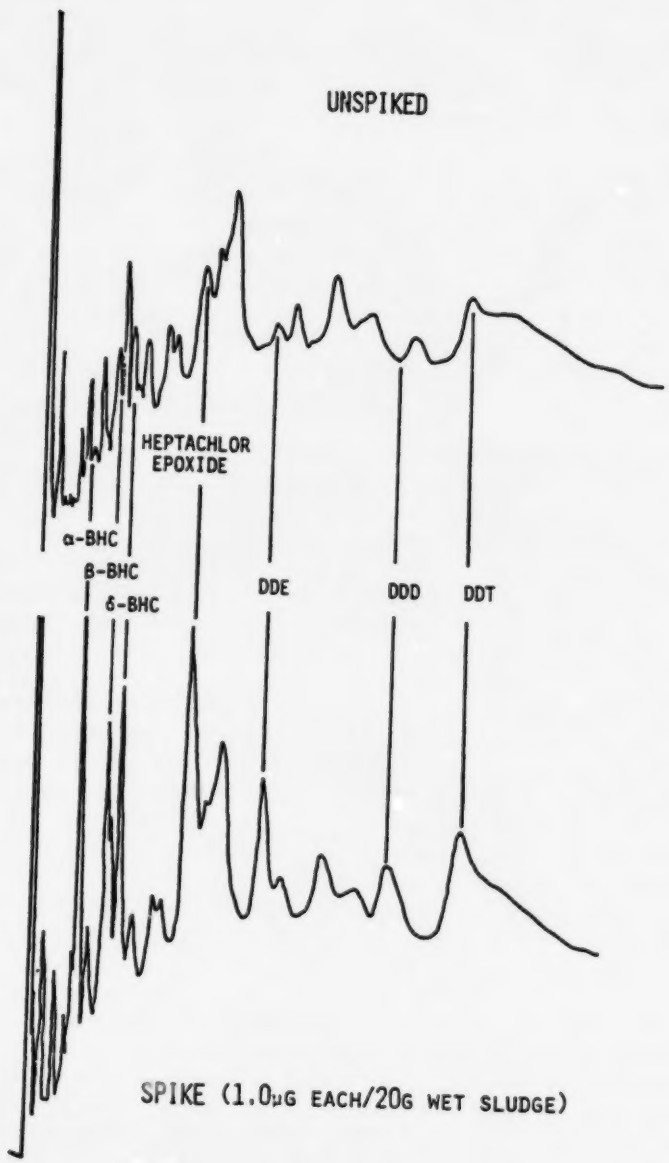

Figure 6. Chromatogram of Group 1 in primary sludge.

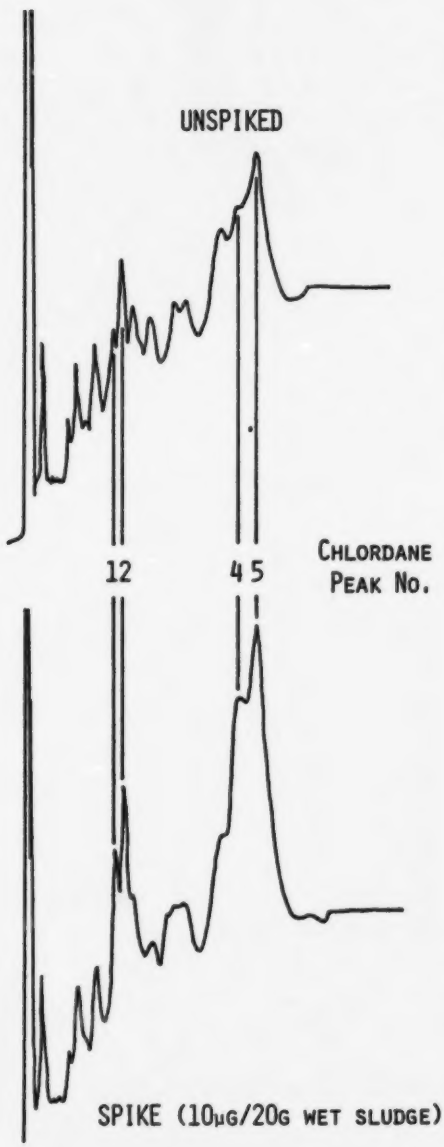

Figure 7. Chromatogram of Group 3 in primary sludge.

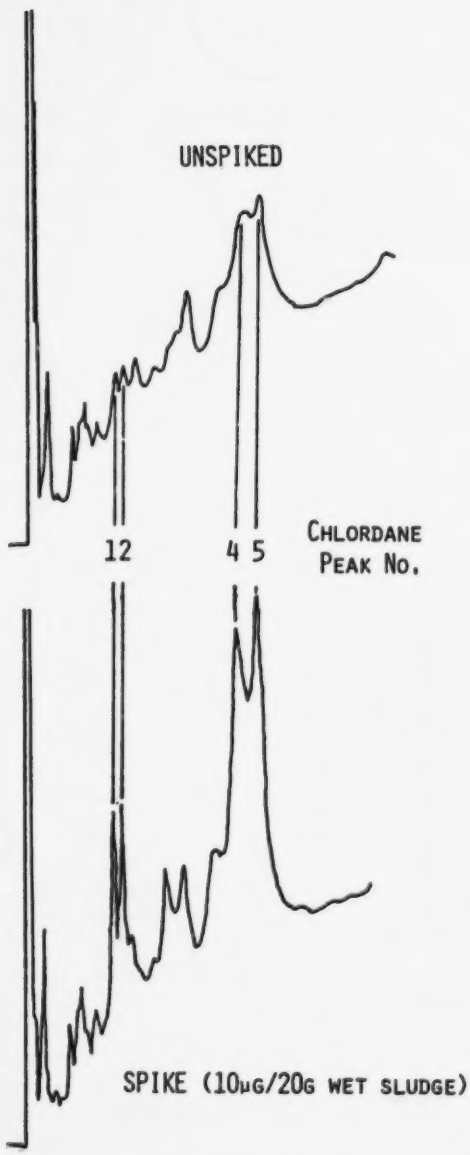

Figure 8. Chromatogram of Group 3 in combined sludge.

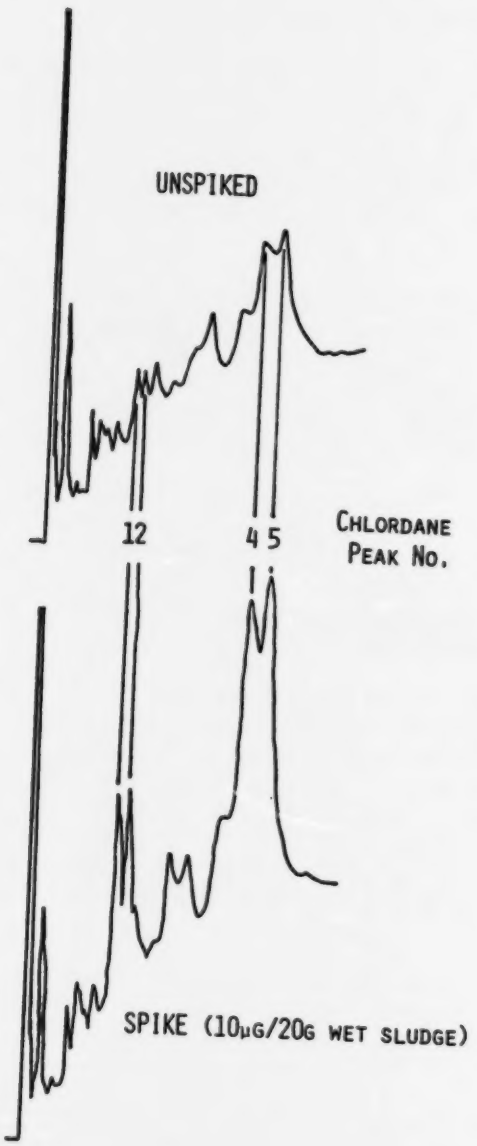

Figure 9. Chromatogram of Group 3 in digested sludge.

for the primary sludge. In contrast, recoveries of 98 and 92 percent, respectively, were obtained at the 0.5 and 1.0 level using the method on the San Antonio primary sludge. Endosulfan II was not recovered well from the combined sludge in comparison to the remaining matrices. This appears not to be related to the background level observed.

Comparative chlordane recoveries are made using only numbered peaks, 2, 4, and 5. In the San Antonio sludge, quantitation was feasible on peaks 2 through 5, while on the remainder, only peaks 1, 2, 4, 5 were usable, and the comparison is made on those peaks in common. This does point out an additional problem area, as it may be difficult to determine which of the compounds will persist in any given matrix and to therefore define a chlordane result.

In general, for the multicomponent formulations of Groups III, IV and V less complete recovery was observed for these sludges than for the previous effort using the San Antonio sludge. In all four matrices, however, the recoveries for the various peaks were inconsistent, and no pattern existed where one or more of the studied peaks gave the highest recovery in all matrices. This further complicates the quantification of the substance.

Groups IV, toxaphene, the individual peak recoveries were inconsistent in these sludges as they were in the San Antonio sludges. The recoveries were higher and more consistent for the higher spike level. No one peak could be singled out as being indicative of the true concentration of toxaphene and it would require a much more extensive study to determine which peaks, if any, could be used to quantify toxaphene.

The results for Group V (AR 1260) were similar to those for chlordane in that not all peaks were quantifiable and there was generally no consistency among the peaks as to the measured recovery. There was essentially no recovery of AR 1260 in the primary sludge at the low spike level but good recovery at the higher level. The most consistent results were obtained on the combined sludge at the high level, but the recovery was only around 30-35 percent. As in most other groups, the overall recoveries obtained were neither as high nor as consistent as those from the San Antonio sludge previously studied.

The study then proceeded to the testing of other phases of the method in an attempt to improve the overall reliability.

CLEANUP PROCEDURES

The method most widely applied to the cleanup of pesticides has been chromatographic fractionation on Florisil. Florisil cleanup has been used extensively in this laboratory in a modified form. The activity of the Florisil is reduced by the addition of 5% water and two fractions are eluted with 6% and 50% diethyl ether in hexane as opposed to three in the usual method.

TABLE 7. EXTRACTION EFFICIENCY OF SINGLE COMPONENT PESTICIDES

Sludge Type	Spike Level µg/l	Average % Recovery *													
		Group I							Group II						
		α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT	γ-BHC	Hept.	Aldr.	End.I	Diel.	Endr.	End.II	Endr. Ald.
Cincinnati Primary	0†	0	.30	.28	.24	.12	0	.70	.19	23	.15	.56	.19	.57	.28	0
	15	88	37	66	91	71	50	75	0	45	30	0	37	0	8	0
	50	83	48	60	73	62	59	53	57	68	55	31	71	61	104	22
Dayton Combined	0†	.04	.19	.12	.12	.03	0	.34	0	.07	.02	.16	.02	.25	.23	0
	15	83	56	59	61	53	38	75	79	62	48	38	93	70	42	0
	50	90	84	74	80	68	59	74	93	93	71	59	89	89	65	15
Dayton Digested	0†	.03	.26	.18	.15	.04	0	.40	.04	.06	.04	.26	0	.21	0	0
	15	93	63	73	97	76	67	113	51	48	37	6	73	54	93	0
	50	104	107	116	85	71	70	91	90	71	58	48	79	101	98	0

*Cleanup by Florisil column chromatography.
†ng apparently present in unspiked sludges, "blank" value.

TABLE 8. EXTRACTION EFFICIENCY OF MULTICOMPONENT FORMULATIONS

Sludge Type	Spike Level	Average % Recovery									
		Group III-Chlordane			Group IV-Toxaphene		Group V - Aroclor 1260				
		C-2	C-4	C-5	T-1	T-2	A-2	A-3	A-4	A-5	A-6
Cincinnati	0*	3.8	2.9	4.1	0	6.5	12	4.2	4.9	6.7	2.3
Primary	30%	77	91	107	86	48	0	0	0	0	41
	100%	62	110	35	103	106	78	88	89	96	95
Dayton	0*	1.8	3.6	3.9	3.1	0	5.2	2.6	0	3.7	2.3
Combined	30%	52	124	68	85	57	75	81	45	43	85
	100%	55	33	41	72	67	84	37	33	30	34
Dayton	0*	1.7	3.8	4.1	0	0	2.9	0	0	2.9	1.1
Digested	30%	52	51	29	56	29	85	107	50	51	73
	100%	41	48	45	115	103	55	64	45	42	63

* ng apparently present in unspiked sludge, "blank" value.

Extracts were made in the usual manner of sludges spiked with Groups 1 and IV and two of each were fractionated on the activated Florisil PR and 5% water-deactivated Florisil PR. The fractions were than analyzed in the usual manner. Inspection of the average recoveries from the two methods shown in Table 9 readily indicates there is no great difference between the two methods. The only differences noted were qualitative during the elution. The colors observed in the water-deactivated Florisil PR column were more pale and extended almost all the way down the column; whereas, the colors were darker and more distinct and extended only halfway down the activated Florisil PR column.

In an effort to determine how much interfering material was being removed by Florisil cleanups, extracts of three unspiked sludges were fractionated on Florisil columns, the fractions were evaporated to dryness, and the residue was weighed. The total extractable residue was determined for each sludge and the material eluted in each fraction was calculated as a percent of the total extractable residue. The difference between total recovered and total extractable residue was the amount removed by the Florisil column. The results of this experiment shown in Table 10 indicate that a maximum of 14% was removed from the organic extractables of Cincinnati primary sludge. The remainder is eluted in the two fractions retained for analysis. The larger part of it is eluted in the less polar 6% ether fraction which would also contain 15 of the 17 polychlorinated compounds. The cleanup effect is simply a change in relative amounts of analyte interferences. The interfering compounds apparently are similar enough in their properties to the chlorinated compounds in that they resist separation based on the adsorption and partitioning properties of the Florisil chromatographic system.

The interfering compounds are postulated to be hydrocarbons of higher molecular weight such as paraffins, lipids, oils, and greases. These compounds should for the most part be of greater molecular size than the polychlorinated hydrocarbons, hence, should be amenable to separation by molecular size exclusion chromatography also known generally as gel permeation chromatography. As will be discussed later, the Florisil cleaned up extracts proved to contain too much interfering material and gas chromatographic/mass spectrometric determinations could not be made. The use of gel permeation materials was then explored in an attempt to provide cleaner extracts for GC/MS analyses.

Sephadex LH-20, a dextran gel, was used in the first system evaluated. Cyclohexane was used as the mobile phase. LH-20 has strong adsorptive properties in addition to the size exclusion characteristics especially during the chromatography of aromatic and polycyclic compounds. Initial experiments with polychlorinated standard mixtures resulted in recoveries of less than 40% for most of the compounds. Some were completely lost, apparently adsorbed on the column, requiring a more polar solvent to elute them.

Bio Beads S is a series of porous styrene-divinyl benzene copolymer beads used as a gel permeation material and is much less adsorptive than LH-20. Bio Beads S-X2 gel, which was used for the additional GPC determinations, has a molecular weight operating range from 100 to an upper limit of 2700, the

TABLE 9. COMPARISON OF CLEANUP ON TWO FLORISIL ACTIVITY GRADES

	Average % Recovery														
	Group I							Group IV		Group V					
Sorbent	α- BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT	T-1	T-2	A-1	A-2	A-3	A-4	A-5	A-6
Deactivated Florisil	72	83	81	75	59	56	119	72	60	40	56	48	36	48	56
Activated Florisil	70	77	78	71	57	51	118	68	58	47	63	54	40	54	63

TABLE 10. DISTRIBUTION OF EXTRACTS IN FLORISIL CLEANUP FRACTIONS

Sludge Type	Total Residue mg(%)	Total Extracted* mg(%)	Amount in Eluant Fraction mg(%) ♦		Amount Removed mg(%) †
			6% Ether	50% Ether	
Dayton Digested	1170 (5.8)	124.6 (10.6)	101.3 (81.9)	11.3(8.6)	12.5 (9.5)
Dayton Combined	1265 (6.3)	105.8 (8.3)	84 (80.1)	8.6 (7.4)	13.4 (12.5)
Cincinnati Primary	1240 (6.2)	143.3 (11.5)	110.8 (77.3)	12.5 (8.7)	20.0 (14)

* From residue

† based on solids content

♦ based on total extracted

molecular weight exclusion limit. Cyclohexane was used as the mobile phase and the polychlorinated compounds were found to elute in fractions 2-6 when six 25-ml fractions were collected. The multicomponent groups tended to elute in broad bands over two or in some cases three fractions.

The commercial literature included with the S-X2 beads suggested that a more active solvent would elute the polychlorinated compounds in a narrower band. The liquid phase was changed to DCM since it is not only more active than cyclohexane but it is more volatile and solutions in it can be concentrated more readily. An additional plus was the fact it is used extensively in organic pollutants analyses.

Standard solutions of the five groups were chromatographed on the S-X2. The Group I and II separations had 1 µg of each compound placed on the column, and for the others there was Group III, 10 µg; Group IV, 50 µg; Group V, 20 µg. Initial studies indicated much of the background interference was eluted in the first 85-90 mL DCM coming off the column and the pesticides/PCB's were in the next 25 mL. Accordingly, the first 85 mL eluted from the columns was discarded, the next 5 mL was caught as fraction 1 and the next 25 mL as fraction 2.

Each of these fractions was concentrated and chromatographed. Percent recovery was calculated for each peak used for these determinations based on the known amount of reference material placed on the column. These results are shown as Table 11. Except for δ-BHC and endrin aldehyde, the recoveries are adequate and quite acceptable. Based on some limited reruns, the results apparently are repeatable and exhibit good precision.

In order to compare efficiences of the cleanup methods for removing interferences, four sludges were extracted in the usual manner. The extracts were taken to dryness under a stream of pure, dry inert gas and weight of extractable organics was determined. The residues were then taken up in DCM and transferred to gel permeation columns and eluted with DCM. The first 90 mL which normally would be discarded was caught and retained as Fraction A and the next 25 mL which normally would contain the pesticides and PCB's was retained as Fraction B. The two fractions were evaporated to dryness and residue weights determined. The percentage recovery was calculated for each fraction and the results of these determinations are compiled as Table 12.

Comparison of these results with those of Table 10 makes it obvious that gel permeation chromatography removes 5-9 times the material that Florisil cleanup does. Gel permeation actually takes 65-85% of interfering substances out of the analytical system. The Florisil can only claim to remove 9-15% of background substances. What this means in terms of the determinative step in the analytical method is that in the case of the Dayton combined sludge, when 1 ng of a pesticide or PCB component is injected into the gas chromatograph, 47,500 ng of other substances are injected along with it. For this same sludge extract cleaned up by Florisil fractionation, the ratio is 1 ng analyte to 84,000 ng "background" which is an increase in background by a factor of two. Apparently gel permeation chromatography provides a better clean-up than does Florisil chromatography but there is still a significant amount of material that could be removed to enhance the analyses.

TABLE 11. RECOVERY OF REFERENCE COMPOUNDS FROM GEL PERMEATION CHROMATOGRAPHIC CLEANUP METHOD

	Percent Recovery															
	Group I.							Group II								
Fraction *	α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT	γ-BHC	Hept.	Ald.	End.I	Diel.	Endr.	End.II	Endr. Ald.
2	1	0	0	7	1	3	2	0	0	0	1	0	0	0	4
3	80	86	47	81	89	83	82	75	81	82	83	85	85	84	69

	Group III - Chlordane				Group IV-Toxaphene			Group V - Aroclor 1260					
	C-1	C-2	C-3	C-4	T-1	T-2	T-3	A-1	A-2	A-3	A-4	A-5	A-6
2	1	1	2	2	2	1	1	1	2	1	1	2	1
3	80	81	83	84	91	93	91	89	88	90	90	89	90

* First 85ml discarded, next 5ml = fraction 2, final 25ml caught = fraction 3; DCM eluting solvent.

TABLE 12. COMPARISON OF GEL PERMEATION CHROMATOGRAPHIC CLEANUP EFFICIENCIES APPLIED TO VARIOUS SLUDGES

Sludge Type	Total Residue mg (%)	Total Extracted * mg (%)†	Amount Recovered in Bio Beads Fractions mg (%) ∓		Amount Lost mg (%)
			Fraction A	Fraction B	
San Antonio Digested	720 (3.6)	79.0 (11.0)	51.9 (65.7)	25.9 (32.8)	1.2 (1.5)
San Antonio Primary	760 (3.8)	96.0 (12.6)	78.8 (82.1)	15.5 (16.0)	1.7 (1.8)
Cincinnati Primary	1240(6.2)	177.0(14.3)	124.8(70.5)	35.6 (20.1)	16.6 (9.4)
Dayton Combined	1180(5.9)	151.2(12.8)	95.9 (63.4)	47.5 (31.4)	7.8 (5.2)

* from 20g wet sludge

† based on solids content

∓ based on total extracted; the total amount recovered subtracted from the total extracted represents the amount "lost" to the method.

Fraction A = first 90ml which would normally be discarded;
Fraction B = next 25ml which contains any pesticides/PCB's present

The recoveries of compounds from GPC shown in Table 11 are as good as or better than any acquired up to that time. The losses were cause for concern though because any compounds being adsorbed on the gel would be subject to breakthrough at any time and would affect results of later determinations. The only other place these compounds could have been lost was through volatilization during evaporation of the solvent. In an attempt to learn where these losses were occurring, 1 mL of spiking solutions containing Group I and Group V were added separately to duplicate 75 mL portions of DCM. The solutions were then carried through all of the solvent evaporation steps that would normally occur in an analytical sequence. These steps are:

1. Evaporate in a Kuderna-Danish apparatus
2. Take down to 2-3 mL under a stream of pure, dry inert gas
3. Rinse into another Kuderna-Danish apparatus with DCM to a volume of 25 mL DCN, add 5-10 mL hexane
4. Evaporate as usual, add 100 mL hexane
5. Evaporate and transfer to a vial
6. Dilute to 10 mL and analyze as usual.

The recoveries of each component in these solutions were calculated in the usual manner and are listed in Table 13.

The comparison of these data with those of Table 11 suggests that most, if not all, of the losses can be laid to the evaporative processes that cause volatilization of the pesticides and PCB's. These losses are probably diminished somewhat in a sludge extract because the high boiling substances act as a "keeper" that serves to decrease volatilization losses of the poly-chlorinateds. Loss of the compounds would then be through some other mechanism such as adsorption or reaction.

A series of experiments was conducted to determine efficiency of the overall analytical method. A Tekmar Tissuemizer homogenizing apparatus became available at this time and replaced manual shaking to mix the sludge and extracting solvent. The homogenizer probe was inserted in the centrifuge tube and the sludge and solvent were mixed at 60 percent on the homogenizer speed control for one minute. The probe was then raised above the extraction mixture and washed off with hexane applied from a Teflon squirt bottle.

The extracts were cleaned up by GPC followed by mercury removal of elemental sulfur. The extracts were analyzed by electron capture gas chromatography. After subtracting "blank" response of the unspiked sludge. percent recoveries were calculated for each component of the pesticides and PCB. Unspiked sludges were run in duplicate and the spiked sludges in triplicate. A summary of the results is shown in Table 14.

TABLE 13. RECOVERY OF REFERENCE COMPOUNDS SUBJECTED TO EVAPORATION PROCEDURES

	Percent Recovery - Group I						
	α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT
Sample 1	73	73	35	75	76	77	68
Sample 2	81	85	43	86	87	88	77
Average	77	77	39	81	82	83	73

	Percent Recovery Group IV - Aroclor 1260					
	A-1	A-2	A-3	A-4	A-5	A-6
Sample 3	78	84	86	86	84	84
Sample 4	78	85	86	86	85	85
Average	78	84	86	86	84	84

TABLE 14. RECOVERY OF REFERENCE COMPOUNDS FROM THE HOMOGENIZATION EXTRACTION AND GEL PERMEATION CHROMATOGRAPHIC CLEANUP OF DAYTON DIGESTED SLUDGE EXTRACTS

Spike Level †	Average Percent Recoveries* ± Standard Deviation						
	Group I						
	α-BHC	β-BHC	δ-BHC	Hept. Epox.	DDE	DDD	DDT
0	.02 ± .01	.33 ± .07	.32 ± .02	.14 ± .01	.04 ± .01	.06 ± .01	.29 ± .01
A	94 ± 28	72 ± 50	62 ± 34	87 ± 57	83 ± 10	90 ± 39	97 ± 98
B	92 ± 3	76 ± 7	81 ± 7	85 ± 8	89 ± 12	95 ± 15	92 ± 25

	Group II							
	γ-BHC	Hept.	Aldr.	Endo.I	Diel.	Endr.	Endo.II	Endr. Ald.
0	0	.09 ± 0	0	0	0	0	0	0
A	64 ± 8	51 ± 12	60 ± 2	112 ± 20	85 ± 28	43 ± 24	82 ± 35	40 ± 17
B	67 ± 8	63 ± 8	60 ± 2	67 ± 7	80 ± 10	75 ± 15	77 ± 10	19 ± 3

	Group III - Chlordane				Group IV - Toxaphene		
	C-1	C-2	C-4	C-5	T-1	T-2	T-3
0	3.7 ± .2	2.4 ± .3	4.5 ± 1	4.9 ± 1	2.5 ± .5	1.5 ± 0	0
A	75 ± 27	68 ± 17	94 ± 32	87 ± 25	88 ± 32	85 ± 24	84 ± 32
B	81 ± 17	78 ± 12	83 ± 13	82 ± 12	88 ± 18	83 ± 13	85 ± 18

	Group V - Aroclor 1260					
	A-1	A-2	A-3	A-4	A-5	A-6
0	1.0 ± .5	2.9 ± .7	1.0 ± .8	0.8 ± 0	2.1 ± 1	0.7 ± .2
A	69 ± 12	81 ± 5	79 ± 15	76 ± 25	85 ± 7	83 ± 12
B	72 ± 20	73 ± 22	72 ± 12	71 ± 27	76 ± 24	78 ± 20

† Spike in wet sludge; A = 30% and B = 100% of nominal value, Groups I and II = 50 µg/l, Group III = 500 µg/l, Group IV = 2500 µg/l and Group V = 1000 µg/l.

* Unspiked recoveries in nanograms (ng); DCM eluting solvent.

Groups I and II, as in other experiments, exhibit a greater degree of reliability at the higher spike level; the results are more consistent and the confidence limits narrow at this level. Again, the values for DDT and endrin aldehyde are the least reliable in these two groups. Apparently, the least reliable values for these determinations are those for the Group I lower spike level.

The values for Groups III and IV are fairly consistent at both spike levels although the confidence limits span a fairly broad range. It may be possible to arrive at a fairly good estimation of quantity of these formulations in sludge within this range of determinations.

The results for Group V are probably the best we have had for this formulation. The recoveries are very consistent over all six peaks and the range of confidence limits is quite acceptable for a determination of this complexity. Overall, these results, with two or three exceptions, are well within the limits for qualifying this method for screening of municipal sludges.

The removal of elemental sulfur from marine sediment extracts has been practiced successfully in this laboratory for quite some time by the simple addition of metallic mercury to the organic extracts. Because of the potential hazards involved in the continual handling of mercury, two other methods of removing elemental sulfur were briefly investigated.

The methods involved contact of the organic solution with copper metal activated by washing with hydrochloric acid or extraction with a solution of tetrabutyl ammonium sulfite which converts the elemental sulfur to soluble thiosulfate. The activated copper caused a reduction in recoveries of a majority of the reference compounds and this approach was quickly abandoned. The tetrabutyl-ammonium sulfite was more effective in removing sulfur although it caused the endrin aldehyde, which is poorly recovered anyway, to be lost completely. The problem with this method is that it introduces several manipulations into an already complex analytical sequence. In our judgement the simplicity of the mercury removal method strongly favors its use. Highly experienced laboratory personnel are required for pesticides analyses and, as such, should be thoroughly familiar with the precautions necessary for the safe handling of mercury.

CONFIRMATION OF IDENTITIES

The confirmation of identity of polychlorinated pesticides and biphenyls extracted from municipal treatment plant sludges reported here can at best be considered in the preliminary phases of study. The complexity and the variety of extractable organic compounds in sludge make the isolation of molecular information a formidable task, even with the sophisticated computer data handling techniques now available.

GC/MS was run on an extract of Dayton digested sludge spiked with the Group I compounds at the level of 0.3 µg of each compound in 20g wet sludge. The extract had been cleaned up by Florisil fractionation and GPC on S-X2 with cyclohexane eluant. The total ion chromatogram (TIC) of fraction 3 is

shown in Figure 10. The elution points for the seven compounds in Group 1 are shown on the chromatogram. Although it is known that these compounds were spiked there is no unequivocal retention time match for any of them.

Single ion chromatograms were made at m/e values characteristic of the individual compounds. The reconstructed single ion chromatogram of m/e 183 is shown as Figure 11. This ion, in combination with those at 109 and 181 is characteristic of the BHC compounds. RSIC's at m/e's 109 and 181 confirmed that only the α-isomer is present in fraction 3; the β- and δ-isomers were found in fractions 5 and 6 by this technique.

The RSIC's at m/e's 246, 248 and 176 indicated the presence of DDE at Spectrum Number 300. The mass spectrum at this point was extracted and cleaned up by subtraction of appropriate background spectra and is shown in Figure 12. This spectrum was then compared with that of an authentic standard shown as Figure 13. Although there is still some contribution from background the match of ion clusters at m/e 176, 246, and 318 is strong evidence to consider this compound identified as p,p'-DDE. This procedure was used for each of the compounds in all five groups and confirmation was obtained for each. The multicomponent formulations present special problems because of the many overlapping compounds and confirmation is difficult but possible at the 30% of nominal value concentration level.

The unspiked digested sludge was searched for the various compounds in the five groups by generating RSIC's of the various characteristic ions. This suggested the possible presence of several of these compounds, notably the BHC's, DDE, DDT, chlordane and an Aroclor possibly 1254 or 1260, but at the very low levels at which they are present it was not possible to generate spectra that could be matched with those of the reference standard materials. With extensive use of RSIC's and the calculation of relative intensities, it should be possible to confirm the presence of these compounds. This approach would take a great deal of time and is not within the scope of this program. Work continues on characterization by GC/MS of the various sludges used on this program.

Clean-up on Bio-Beads with DCM as the eluant has been included as part of the proposed method in the Appendix. DCM as the eluant provides a single extract which is adequate for quantitative determination by GC/ECD and on some sludges is satisfactory for confirmation by GC/MS. If the interferences in the DCM extract do not permit GC/MS confirmation, the six fraction cyclohexane procedure should then be applied to the DCM extract before GC/MS confirmation. Cyclo-hexane as the eluant apparently minimizes the background interferences to a somewhat greater extent than the DCM procedure and allows confirmation of organic identity by GC/MS at lower levels on difficult sludges. The need for the alternative approach is being further tested as work on this contract continues.

Size exclusion chromatography on Bio-Beads proved an excellent means of cleaning up sludges for quantitative determinations by GC/ECD. Although qualitatively, the chromatograms do not appear much different from those of extracts cleaned up by Florisil column chromatography, gravimetric

determinations proved the Bio-Beads removed a great deal more material. Because of their high boiling points, the substances not removed by Florisil would be expected to deposit on the gas chromatographic column and cause performance to deteriorate steadily and decrease column life. Thus clean up on Bio-Beads is the recommended approach to quantitative determination of polychlorinated pesticides and biphenyls in municipal sludges.

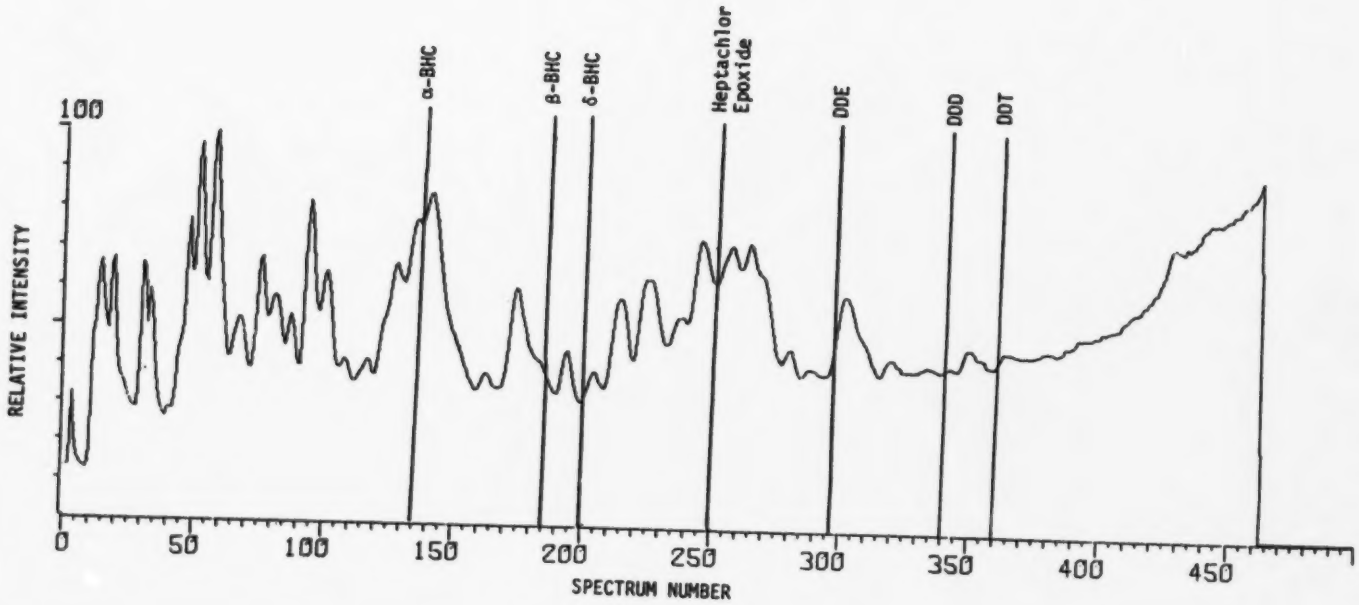

Figure 10. Total ion chromatogram of GPC fraction 3 from organic extract of Dayton digested sludge spiked with Group I polychlorinated pesticides.

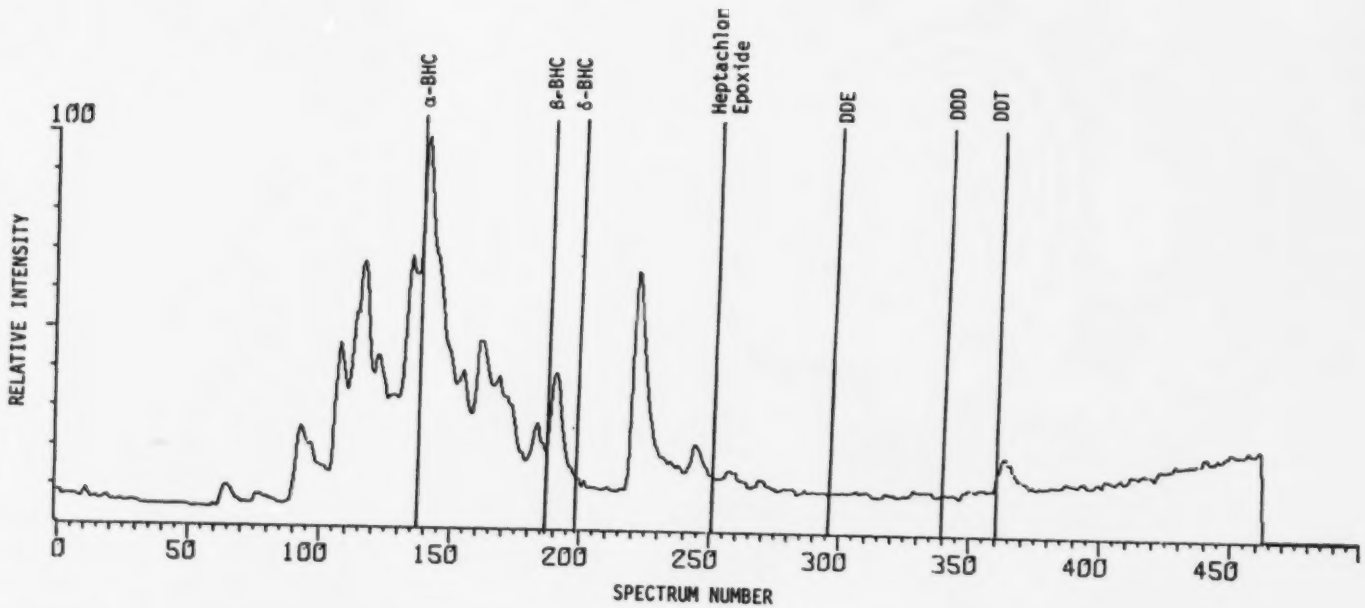

Figure 11. Reconstructed single ion chromatogram for m/e 183 GPC fraction 3 from organic extract of Dayton digested sludge spiked with Group I polychlorinated pesticides.

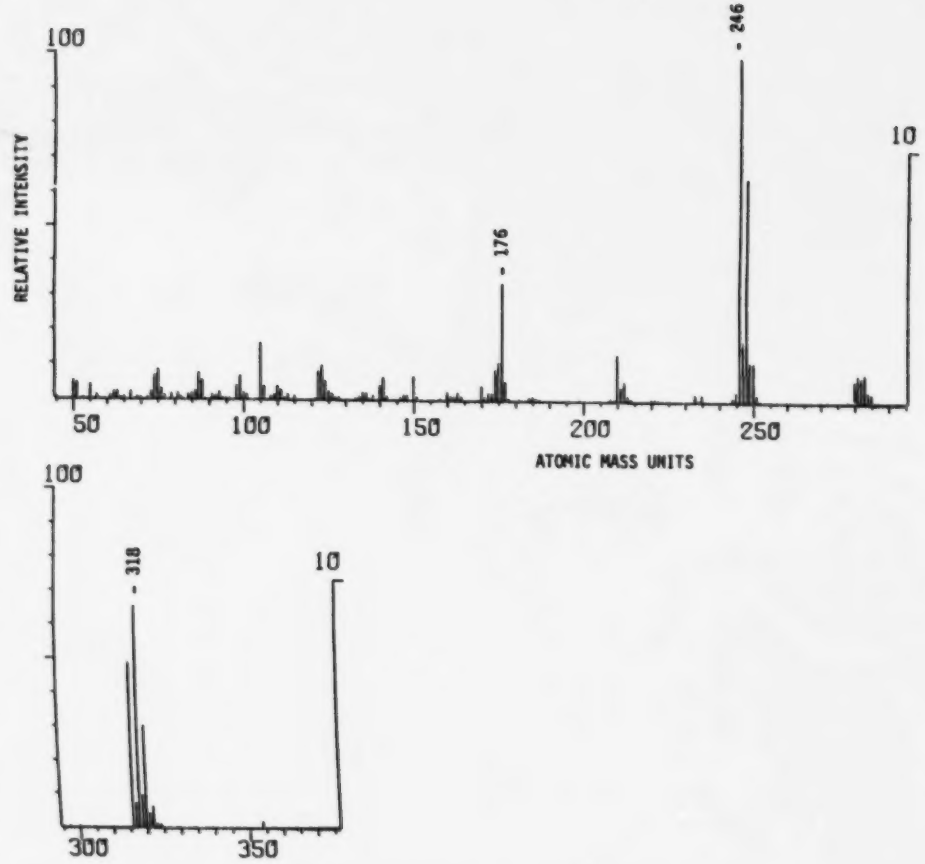

Figure 12. Corrected mass spectrum of reference standard, p,p'-DDE.

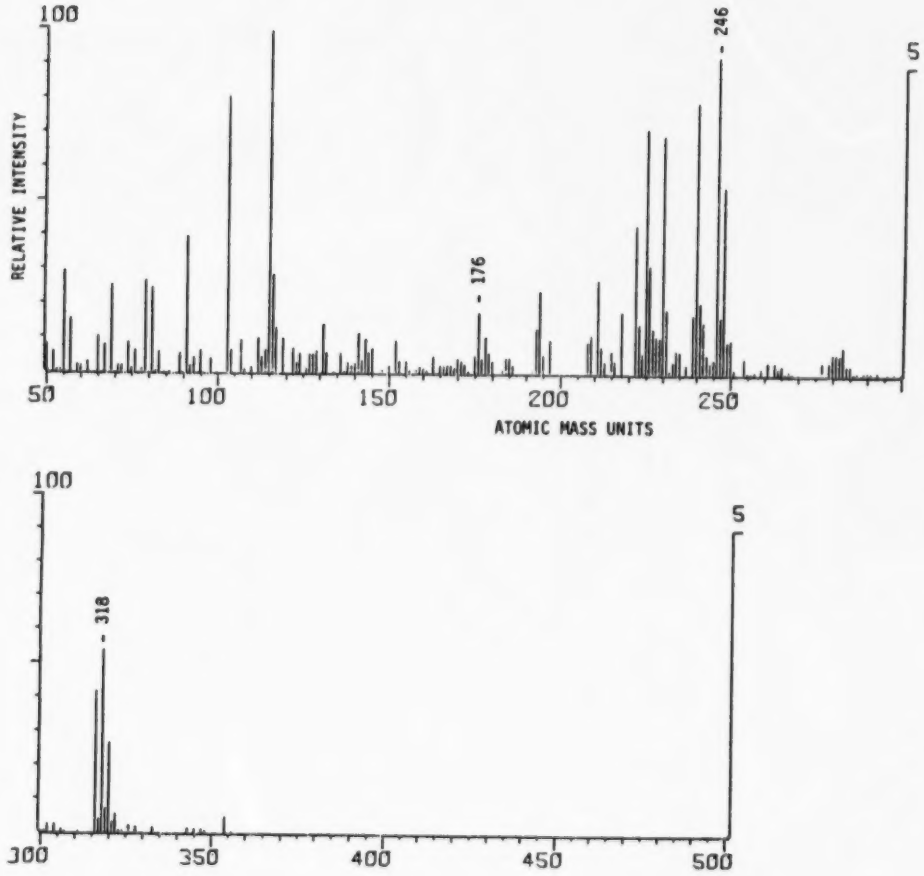

Figure 13. Corrected mass spectrum of compound indicated to be p,p'-DDE in GPC fraction 3 of organic extract from Dayton digested sludge spiked with Group I compounds.

APPENDIX

MX00 - METHOD FOR THE ANALYTICAL DETERMINATION OF CHLORINATED PESTICIDES AND POLYCHLORINATED BIPHENYL IN MUNICIPAL SLUDGE BY GAS CHROMATOGRAPHY WITH ELECTRON CAPTURE DETECTION

X10 Scope and Application

X11 This method covers the determination of various chlorinated hydrocarbon pesticides and a polyclorinated biphenyl by gas chromatography and electron capture detection. The complete list of compounds included in this procedure is provided in Table X11A.

X12 This method is applicable to the analytical determination of these compounds in the sludge from municipal waste treatment plants. It can be used to meet the monitoring requirements of the National Pollutant Discharge Elimination System (NPDES). As such, it presupposes a high expectation of finding specific compounds of interest. If the user is attempting to screen samples for all of the compounds in Table X11A, reference should first be made to Section X81 for general guidance.

X20 Summary

X21 The method offers limited analytical alternatives; their use is dependent on the specific compound(s) of interest and the nature and extent of interferences. The procedure describes the use of liquid-liquid extraction with a moderately polar solvent mixture to minimize coextraction of interferences while efficiently recovering the compounds of interest. Selected general purpose cleanup procedures are included to aid in the elimination of interferences. Chromatographic conditions are suggested for the qualitative and quantitative determination of the compounds.

X22 This method is recommended for use only by experienced pesticide analysts or under the close supervision of such qualified persons. All personnel that use this method should be thoroughly familiar with the principles of analytical pesticide determinations in the manual, "Analysis of Pesticide Residues in Human and Environmental Samples," Ed.: J. F. Thompson, June 1977.

X30 Apparatus and Reagents

X31 For sample extraction, Section X50:

X31.1 Neutralizing solutions - H_2SO_4/H_2O (1:2) and 10% (w/v) aq NaOH.

X31.2 Pipet, transfer, 50mL.

X31.3 Kuderna-Danish (K-D) glassware -

a. Synder column - three ball macro

b. Evaporation body - 500mL and 250mL

c. Receiver ampul - 10mL, graduated

d. Ampul stoper

e. Boiling stones, Hengar, plain, 10 mesh, for micro determinations

X31.4 Centrifuge tube, screw cap, 250 or 200mL

X31.5 Teflon film, 1 mil, 4 inches wide by 100 foot long, cut to 4" squares

X31.6 Extracting solvent - hexane, dichloromethane, acetone (83:15:2), all solvents pesticide grade, redistilled in glass, if necessary.

X31.7 Sodium sulfate - pesticide grade, granular, anhydrous (extracted 48 hours with dichloromethane, air dried and conditioned at 100°C for 18 hours, wash with hexane just prior to use).

X31.8 Stirrer - paddle with variable speed motor.

X31.9 Salt solution - NaCl, saturated in purified water; if required the reagent grade NaCl is cleaned up in the same manner as the Na_2SO_4.

X31.10 Steam bath , with drain so condensate does not collect in the bath.

X32 For quantitation, Section X60:

X32.1 Gas chromatograph - equipped for on-column injection.

X32.2 Glass column, 1.8m long x 4mm ID packed with 1.5% SP-2250/ 1.95% SP-2401 on 100/120 mesh Supelcoport.

X32.3 Recorder - potentiometric strip chart (25cm) compatible with detector.

X32.4 Syringe - 10µL volume. Additional sizes will be required.

X32.5 Reference materials - assayed quantity of compound(s) of interest, 1µg/µL stock solution in isooctane (except β-BHC in acetone) and appropriate dilute solutions.

X32.6 Computer - integrating, interfaced to the gas chromatograph, optional.

X32.7 Carrier gas - zero grade, high-purity, organic and oxygen-free, filtered through 13X molecular sieve and a catalytic oxygen scrubber.

X33 For removal of interferences, Section X70:

X33.1 Gel Permeation Chromatography (X72)

a. Chromatographic column - Pyrex (22 mm ID x 400 mm long) with coarse fritted disc at bottom and a stop-cock - equipped stem with a teflon plug.

b. Bio-Beads - Grade S-X2 (200-400 mesh) a spherical, porous styrene-divinylbenzene copolymer with 2% crosslinking, available from BIO-RAD laboratories, Richmond, California. Before use swell the gel by soaking in dichloromethane.

c. Dichloromethane - pesticide quality, redistilled in glass if necessary.

d. Cyclohexane- pesticide quality, redistilled in glass if necessary.

e. Inert gas - zero grade, high purity, organic free, dry; as a precaution should be filtered through 13X molecular sieve.

f. Hexane - pesticide quality, redistilled in glass, if necessary.

X33.2 Sulfur removal (X73) -

a. Mercury - triple distilled
b. Shaker - reciprocating, variable speed
c. Mixer - Vortex tube.

X40 Sampling and Preservation

X41 Samples should be collected in quantities of 1000g or more and stored in glass containers equipped with an inert liner (teflon or foil) in the cap. The container should be prewashed, solvent rinsed, air- and oven-dried before use to minimize interferences. Conventional sampling practices should be followed, except that the bottle must not be prewashed with sample before collection. Since municipal sludge is such a complex mixture, usually containing oily materials, there is a significant probability that these oils would adhere to the sample container. These oils would be expected to selectively dissolve chlorinated pesticides thereby changing concentrations in the sample.

X42 Generally, samples should be analyzed as soon as possible after sampling. Since this may not be ordinarily possible, the sample should be refrigerated or iced immediately after collection and maintained at ≈5°C until extraction which should not be delayed any longer than absolutely necessary. Samples stored for more than seven (7) days are suspect. An acceptable alternative to storage of samples is immediate extraction and cold storage of the extract in the dark. Decomposition in the extracts can be evaluated by storing spiked controls along with the samples.

X50 Sample Extraction

X51 Stir the sample and allow to come to room temperature. Neutralize to pH 7.0±0.5 with dilute H_2SO_4 or NaOH solution. While the sample is being stirred to keep the solids distributed, withdraw and weigh a 20g analytical sample. Transfer the analytical sample to a 200mL centrifuge bottle containing 20mL saturated NaCl solution.

X52 Add 60mL extracting solvent, cover the mouth of the centrifuge bottle with teflon film and affix the screw cap; shake for two (2) minutes or agitate one (1) minute with a high speed, immersible probe homogenizer.

X53 Place the bottle(s) in a refrigerated centrifuge maintained at 15°C and spin at 500XG for 20 minutes.

X54 Remove the extracting solvent (top layer) from the centrifuge bottle with a 50mL pipet and pass it through a chromatographic column containing 10-15mm of anhydrous Na_2SO_4 and collect it in a 500mL K-D flask equipped with a 10mL receiver ampule.

X55 Add 60mL extracting solvent to the centrifuge bottle and repeat the procedure adding the extract to that in the K-D apparatus.

X56 Perform the extraction procedure a third time in the same manner.

X57 Add a clean boiling chip to the extract; attach a three-ball Synder column to the evaporator and support the apparatus in a steam bath. Concentrate the extract to 1-2mL in the ampule (10-20 minutes) and remove from the steam bath. The condensed vapor will drain into a final volume of 5-10mL in the ampule.

X58 Remove the Synder column and rinse the extractor flask into the ampule with 1-2mL hexane. A 5mL syringe is recommended for this operation.

X59 Stopper the ampule and store ≈5°C in a dark location while awaiting chromatography.

X60 Quantitation

X61 Quantitative measurements called for by this method are made by gas chromatography and electron capture detection. The electron capture detector provides some selectivity to certain molecules such as alkyl halides, conjugated carbonyls, nitriles, nitrates and polycyclic aromatics and response is so low as to be virtually nonexistent to hydrocarbons, alcohols, unconjugated carbonyls and similar molecules. Response is the greatest to halogen and nitroso compounds and increases sharply for multiple halogen substitution, thereby enhancing selectivity to a great degree for the compounds listed in Table X11A. Use of the electron capture detector requires cleanup procedures prior to quantitation to remove organic interferences and elemental sulfur. The column packing recommended for use with this method provides resolution adequate for the quantitative determination of the compounds listed in Table X11A.

X62 The electron capture detector must be operated within its linear response range and at a noise level less than 2% full scale. A final dilution of 10mL extract from a 20g wet sample is optimum' any greater concentration of extract without further cleanup overwhelms the detection system and can render it inoperative for as long as a day.

X63 Calibration and spiking solutions are made from the 1µg/µL stock solutions of the individual standards. Dilutions are made with hexane such that an injection of 10µL will contain the appropriate Group I or Group II standard(s) in the amounts of 0.3 and 1.0ng; 3 and 10ng Group III; 15 and 50ng Group IV and 6 and 20ng Group V. Stock solutions are diluted with acetone such that 1mL will contain the appropriate amo' .t of standard(s) for spiking the wet sludge with the desired amount. If the analytical determination is being made of more than one compound in Group I or II the procedure may be simplified by mixing the compounds to make one spiking solution. The amount of compound spiked in 20g wet sludge is 0.3 and 1µg of each of the compounds in Group I and II; 3 and 10µg total of Group III; 15 and 50µg total of Group IV, and 6 and 20µg total of Group V.

X64 Organohalide pesticides - The retention times relative to that of aldrin of the 17 pesticides are listed in Table X64A. The four (4) peaks listed for Group II and three (3) peaks listed for Group IV are the most representative ones noted in the chromatograms of those formulations. It is apparent from these retention times that certain combinations of pesticides will result in overlapping chromatographic peaks; hence, unambiguous identifications cannot always be made. The procedures given in this method for the removal of interferences will only partially resolve these anomalies. In order to obtain data from the quantification of coeluting compounds, it is necessary that an experienced pesticide analyst apply these procedures and others such as column adsorption chromatography and gas chromatography/mass spectrometry (both scanning and single ion monitoring).

X65 Chlorinated hydrocarbons - The relative retention times of five peaks of the polychlorinated biphenyl listed in Table X65A are representative of this multicomponent formulation. The analyst may key on these peaks to determine the presence of AR 1260 in sludge extracts in combination with the comparison to the entire chromatogram of an authentic standard. As in X64, the presence of other chlorinated hydrocarbons which coelute with known compounds in the AR 1260 will increase the difficulty of identification and quantification. In this instance, it will be necessary to use additional GC/MS techniques from this method and others from the scientific literature to enhance the probability of unequivocal identification.

X66 General comments - The determination of any of the chlorinated hydrocarbons within the scope of this method requires the application of an appropriate cleanup procedure such as the gel permeation chromatography technique. This cleanup removes a significant amount of organic extractable substances thereby eliminating them as interferences which would make it virtually impossible to use electron capture detection for the analysis of municipal sludge. The extracts prepared in this manner are suitable for confirmation of identity by GC/MS or, if a cleaner extract is required, the more retentive GPC cleanup (X74) may also be used. Method MX00 has been qualified for one polychlorinated biphenyl, AR 1260, but it is of equal applicability to those listed in Table X66A. The response characteristics for each formulation would have to be determined by gas chromatography/electron capture detection prior to analytical determination in sludge.

X70 Removal of Interferences

X71 The procedures in this section have proven utility in the analysis of muncipal sludges for the determination of polychlorinated hydrocarbons. Gel permation chromatographic fractionation allows the electron capture detector to be used for chromatographic detection as does the removal of elemental sulfur with mercury. GPC also provides cleanup which facilitates confirmation of compound identity by GC/MS; for further fractionation and enhancement of GC/MS the more adsorptive GPC procedure in X74 may be used after cleanup.

X72 GPC provides cleanup by eliminating high molecular weight materials which are not retained on the gel as are the compounds of interest. The response of interferences is decreased so the polychlorinated hydrocarbons may be recognized by electron capture detection.

X72.1 Cover 30-40g Bio-Beads S-X2 with DCM and let stand overnight to swell the polymer.

X72.2 Place a glass wool plug over the fritted disc of the chromatographic column; rinse the column and glass wool with DCM and tap down with a glass rod.

X72.3 Close the stopcock and add enough DCM to cover the glass wool.

X72.4 Add the swollen gel in small portions to the column while draining excess DCM and tapping the sides of column; continue until a well-packed gel column 30-31 cm in height results.

X72.5 Carefully drain the DCM to just expose the top of the gel; add a 15-20 mm layer of anhydrous, granular Na_2SO_4 and suffient DCM to cover it.

X72.6 Adjust the sample extract volume to less than 3 mL in DCM.

X72.7 Drain the DCM from the column to just expose the top of the gel; quantitatively transfer the extract to the gel bed keeping the volume as small as possible: drain the column to just expose the gel bed below the Na_2SO_4 and close the stopcock.

X72.8 Add DCM to the column to a level of 20-40 mm above the Na_2SO_4; maintain this level throughout the elution.

X72.9 Open the stopcock to begin solvent flow at a rate of ≈3 mL/minute under these conditions.

X72.10 Discard the first 90 mL eluted.

X72.11 Collect the next 25 mL of eluate in a 250 mL K-D flask equipped with a 10 mL ampule.

X72.12 Concentrate the 25 mL eluate of interest to 5-8 mL in the K-D evaporative concentrator, cool, add 100 mL of hexane and concentrate again to 5-8 mL.

X72.13 Quantitatively transfer the concentrate by decantation and three 1 mL washings to a 10 mL volumetric flask and evaporate to less than 10 mL with a stream of pure, dry inert gas. Make the solution to 10 mL with hexane.

X73 Sulfur removal - Most extracts of municipal sludge are expected to contain elemental sulfur which interferes with the gas chromatographic analysis. The sulfur can be removed by shaking a portion of the extract with triple distilled mercury.

X73.1 Quantitatively transfer a 2mL aliquot of the extract to a 5 mL foil- or teflon-lined screw cap vial.

X73.2 Add 0.4 mL triple distilled mercury to the extract.

X73.3 Shake for approximately 10 seconds on a vortex mixer and then for 2 hours on a reciprocating shaker at 3-5 cycles per second.

X73.4 At the end of one hour of shaking, check the condition of the mercury by taking the vial in the hand and swirling in a circular motion. If a bright mercury color is not visable at the bottom of the vial, add 0.2mL mercury and continue shaking; otherwise, continue shaking to the end of the 2-hour period.

X73.5 Allow the extract to settle undisturbed for a minimum of 15 minutes.

X73.6 Withdraw 10µL of the organic extract with a microliter syringe without disturbing the precipitate.

X73.7 Inject the extract into a GC/ECD for quantitative determinations.

X74 Gel permeation chromatography - on Bio-Beads S-X2 with cyclohexane mobile phase, if necessary will fractionate the extract and minimize some of the interferences to allow enhancement of gas chromatographic/mass spectrometric response for configuration of identity of the polychlorinated compounds.

X74.1 Place 25g Bio-Beads S-X2 in a beaker and add 2-3 volumes of cyclohexane. Cover the beaker and let stand overnight.

X74.2 Transfer the swollen gel to a glass, 200mm diameter column equipped with a stopcock. The bed volume is approximately 50cc.

X74.3 Flush the column with 75-100mL cyclohexane under an inert gas pressure of ≈62kPa without letting the gel go dry.

X74.4 Allow the cyclohexane to drain to the top of the gel bed and transfer the remaining 8mL extract without the sulfur removed to the top of the gel bed.

X74.5 Wash the vial and sides of the column 3-4 times with small volumes of cyclohexane allowing the solvent to drain to the top of the gel bed each time.

X74.6 Fill the column with cyclohexane and elute at ≈5mL/min under an inert gas pressure of ≈62kPa.

X74.7 Collect six (6) 25 mL eluate fractions, each in a K-D evaporative concentrator.

X74.8 Evaporate each fraction in a steam bath as in Section X57 to a volume <5mL, being careful not to let the sample do dry.

X74.9 Transfer each concentrate to a 0.5mL foil- or teflon-lined screw cap vial.

X74.10 Concentrate each fraction to ≈0.1mL under a stream of pure, dry inert gas and store refrigerated for GC/MS determinations.

X80 GC/MS Confirmation of Identity

X81 Extracts of sludge from municipal treatment plants will normally contain significant amounts of substances that respond to the gas chromatographic electron capture detector. A positive response at a retention time coinciding with that of the compound formulation listed in Table X11A cannot be considered absolute proof that such a substance is present. Confirmatory identification by gas chromatography/mass spectrometry should be used routinely for the analysis of municipal sludges.

X82 If this method is to be used to survey samples for a large number of compounds, then the verification of identity by GC/MS becomes essential.

X83 Tentative identification of the pesticides and PCB's is made by comparison of the mass spectra of the unknowns with those of authentic standards extracted from spiked sludge and recorded under similar gas chromatographic conditions.

X84 A useful guide for the GC/MS confirmation of identity is the EPA manual, "Sampling and Analysis Procedures for Screening of Industrial Effluents for Priority Pollutants," April 1977.

X90 Quality Assurance and Quantification

X91 The determination of quantities of the analyte substances is done by the "method of additions" using the results of analysis of the unspiked and two spiked sludges as the three data points to construct the calibration curve as shown in Figure X91A. Relatively "clean" sludge will allow the use of peak areas for the measure of response but in most cases peak heights are more reliable and it is recommended they be used unless the reliability of peak area measurements can be demonstrated.

X92 The multicomponent formulations present a problem in that each peak within a chromatogram will be different from the others in response characteristics and it is also quite difficult to get a match between standards and environmental samples. The recommended method of quantification in this instance is to use the sum of the response of peaks which from mass spectrometric and retention data are recognized as belonging to the formulation to construct the calibration curve.

X93 Standard quality assurance practices should be used with this method to the extent necessary to ensure reliable results. Field replicates should be collected to validate the precision of the sampling technique and laboratory blanks and replicates should be analyzed to validate the precision of the analysis. The analysis of fortified samples can be used to validate the accuracy of the analysis.

X94 Sludge from municipal sewage treatment plants is variable in organic content. Because of this, a routine should be followed throughout the program of interspersing samples fortified in the field throughout the real samples for blind analyses in the laboratory.

X100 Calculations and Reporting

X101 The concentration of analyte in the sample is determined as μg chlorinated hydrocarbon per 20g wet sample. Determine concentration in the sample on a dry basis according to the following formula

$$\text{Micrograms/g-Solids} = \frac{C}{P \times W}$$

C = amount of analyte in μg in 20g in sludge.

$$P = \frac{\%\ \text{solids in sludge}}{100}$$

W = weight of sample analyzed in grams.

X102 Report results in micrograms per gram solids without correction for recovery data. All data obtained should be reported.

TABLE X11A. LIST OF SUBSTANCES DETERMINED BY THIS METHOD

Group I	Group II
α-BHC	γ-BHC
β-BHC	Heptachlor
δ-BHC	Aldrin
Heptachlor epoxide	Endosulfan I
p,p'-DDE	Endosulfan II
p,p'-DDD	Dieldrin
p,p'-DDT	Endrin
	Endrin aldehyde

Group III	Chlordane
Group IV	Toxaphene
Group V	PCB, Aroclor 1260

TABLE X64A. RELATIVE RETENTION RATIOS OF ORGANOHALIDE PESTICIDES

Pesticide	Retention Ratio
α-BHC	0.56
γ-BHC	0.69
β-BHC	0.78
Heptachlor	0.84
δ-BHC	0.90
Aldrin	1.00
Heptachlor epoxide	1.46
Endosulfan I	1.82
p,p'-DDE	2.12
Dieldrin	2.21
Endrin	2.66
p,p'-DDD	3.20
Endosulfan II	3.23
p,p'-DDT	3.86
Endrin aldehyde	4.17
Chlordane *	0.84, 1.13, 1.62, 1.75, 3.12
Toxaphene *	2.20, 3.28, 3.97

*These formulations are complex mixtures of many compounds and analytical data will vary among samples. It is essential that the analyst treat these formulations carefully and compare mass spectrometric and retention data often with that of authentic standards.

TABLE X65A. RELATIVE RETENTION RATIOS OF PCB AR-1260

Peak No.	Retention Ratio
1	2.38
2	2.63
3	2.98
4	3.36
5	3.79
6	5.95

TABLE X66A. LIST OF POLYCHLORINATED BIPHENYLS TO WHICH THIS METHOD IS APPLICABLE *

Aroclor 1016
Aroclor 1221
Aroclor 1232
Aroclor 1242
Aroclor 1248
Aroclor 1254
Aroclor 1260
Aroclor 1262

*The polychlorinated biphenyls are multicompenent mixtures which exhibit numerous peaks over a broad retention time range. It is essential to the recognition of these mixtures that the analyst thoroughly familiarize himself with the gas chromatographic and mass spectrometric characteristics of authentic standard mixtures of these formulations.

TABLE X72A. DISTRIBUTION OF POLYCHLORINATED HYDROCARBONS IN FLORISIL CLEANUP FRACTIONS

Eluate	Compound
6% Ethyl ether/94% Hexane	Aldrin, α-, β-, δ-, and γ-BHC, chlordane, p,p'-DDE, p,p'-DDD, p,p'-DDT, heptachlor, heptachlor epoxide, endrin, dieldrin, endosulfan I, toxaphene, PCB.
50% Ethyl ether/50% Hexane	Endosulfan II, endrin aldehyde